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DENDRIMERS IN ANTICANCER TARGETED DRUG DELIVERY: ACCOMPLISHMENTS, CHALLENGES AND DIRECTIONS FOR FUTURE

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Dendrimers are nanoparticles with unique features including globular 3D shape and nanometer size. The availability of numerous terminal functional groups and modifiable surface engineering permit modification of dendrimer surface with several therapeutic agents, diagnostic moieties and targeting substances.

The aim. To enlighten the readers regarding design, development, limitations, challenges and future directions regarding anticancer bio-dendrimers.

Materials and methods. The data base was represented by such systems as Medline, Cochrane Central Register of Controlled Trials, Scopus, Web of Science Core Collection, PubMed. gov, Google-Academy. A search was carried out for the following keywords and combinations: Polypropylene imine (PPI); Poly-L-lysine (PLL); Polyamidoamine (PAMAM); cancer; drug delivery; dendrimers.

Results. High encapsulation of drug and effective passive targeting are also among their therapeutic uses. Herein, we have described latest developments in chemotherapeutic delivery of drugs by dendrimers. For the most part, the potential and efficacy of dendrimers are anticipated to have considerable progressive effect on drug targeting and delivery.

Conclusion. The newest discoveries have shown that the dendritic nanocarriers have many unique features that endorse more research and development.

Keywords: Polypropylene imine (PPI); Poly-L-lysine (PLL); Polyamidoamine (PAMAM); cancer; drug delivery; dendrimers

Abbreviations: PPI – Polypropylene imine; PLL – Poly-L-lysine; PAMAM – Polyamidoamine; PDI – Polydispersity index; SiRNA – Small interfering ribonucleic acid; DOX – Doxorubicin; PTX – Paclitaxel; G4 – Generation 4; DTX – Docetaxel; TZ – Trastuzumab; HER2 – Human epidermal growth factor receptor type 2; FA – Folic acid; HABA – 4'-hydroxyazobenzene-2-carboxylic acid; DSC – Differential scanning calorimetry; rMETase – recombinant methioninase; DAB – 1,4-diaminobutane; scFvs – single chain fragment variables; Ara-C – Cytarabine; GL – Glycyrrhizin.

ДЕНДРИМЕРЫ В ТАРГЕТНОЙ ДОСТАВКЕ ПРОТИВООПУХОЛЕВЫХ ПРЕПАРАТОВ: ДОСТИЖЕНИЯ, ПРОБЛЕМЫ И ПЕРСПЕКТИВЫ ДАЛЬНЕЙШИХ ИССЛЕДОВАНИЙ

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Дендримеры – это наночастицы с уникальными характеристиками, представляющими собой сферическую трехмерную форму и нанометровый размер. Доступность многочисленных концевых функциональных групп и модифицируемая инженерия поверхности позволяет изменить поверхность дендримеров с помощью нескольких терапевтических агентов, диагностических групп и таргетных веществ.

Цель. Ознакомить читателей с дизайном, разработкой, ограничениями, проблемами и перспективами дальнейших исследований противоопухолевых биодендримеров.

Материалы и методы. База данных была представлена такими системами как Medline, Cochrane Central Register of Controlled Trials, Scopus, Web of Science Core Collection, PubMed. gov, Google-Academy. Проведен поиск по следующим ключевым словам и сочетаниям: полипропиленимин, поли-L-лизин, Полиамидоамин – Polyamidoamine (PAMAM); рак; доставка лекарств; дендримеры.

Результаты. Высокая инкапсуляция препарата и эффективное пассивное таргетирование относятся к числу его терапевтических применений. Были описаны последние разработки в области химиотерапевтической доставки лекарств с помощью дендримеров. По большей части, потенциал и эффективность дендримеров, как ожидается, окажут значительное прогрессивное влияние на таргетирование при доставке лекарств. Заключение**.** Новейшие открытия показали, что дендритные наноносители обладают многими уникальными свойствами, которые требуют дополнительных исследований и разработок.

Ключевые слова: полипропилен имин (PPI); Поли-L-лизин (PLL); полиамидоамин (PAMAM); рак; доставка лекарств; дендримеры

Сокращения: PPI – полипропиленимин; PLL – Поли-L-лизин; PAMAM – Полиамидоамин; PDI – Индекс полидисперсности; siRNA – Малая интерферирующая рибонуклеиновая кислота; DOX – Доксорубицин; PTX – Паклитаксел; G4 – Поколение 4; DTX – Доцетаксел; TZ – Трастузумаб; HER2 – Рецептор эпидермального фактора роста человека типа 2; FA – Фолиевая кислота; HABA – 4'-гидроксиазобензол-2-карбоновая кислота; DSC – Дифференциальная сканирующая калориметрия; rMETase – Рекомбинантная метиониназа; DAB – 1,4-диаминобутан; scFvs – Переменные фрагменты одной цепи; Ara-C – Цитарабин; GL – Глицирризин

INTRODUCTION

Chemotherapeutic agents are administered to cancer patients with an intent to inhibit the growth of proliferating cells [1]. However, in many circumstances due to the lower extent of drug delivery, generalized delivery of drug to all parts of the body including areas which do not have tumors and various side effects, the expected aims and goals are not achieved. Nanomedicine is a field of science that deals with therapeutic agents/substances whose average particle size is in the range of nanometers [2]. In comparison to the traditional drug delivery agents including tablets, capsules etc, the design and development of targeted drug delivery systems has gained attention in the recent decades as they offer several advantages over their traditional counterparts [3, 4]. Although chemotherapeutic agents are available in the management and treatment of cancer however they possess numerous side effects and also exhibit weak anticancer activity. Moreover, these traditional systems cannot deliver the drug selectively to tumor interstitium. Novel drug delivery systems are designed keeping in mind the challenges faced by traditional chemotherapeutics and to address the issues related to them. The novel drug delivery systems include polymeric micelles, nanoparticles, liposomes and dendrimers, [5, 6] while some systems have found their way to the market such as Doxil© (liposomes loaded with doxorubicin) and Abraxane© (paclitaxel bound to albumin) [7].

Dendrimers are 3D globular molecules possessing a central core and from that core numerous arms originate with extensive branching [8, 9]. Compounds and conjugates to formulate dendrimers are synthesized sequentially step-by-step which provides uniform and even branching to molecules, specific groups on the surface, low polydispersity index (PDI) and unique molecular size. Hence dendrimers synthesized by stepwise process possess numerous advantages over polymers synthesized in single step. The first reporting of dendrimers was recorded almost 3 decades ago [10, 11], though early exploration only focused on their chemical and physical characteristics and the synthesis steps, and it was since the last decade when researchers started to discover their potential in the field of nanomedicine and other associated biomedical fields. Dendrimers have shown promise in many areas such as in chemotherapy, vaccine development, antivirals, antibacterials, siRNA/gene delivery and various diagnostic applications in medicine and health sector [12–15].

The structure of dendrimers is the key in offering benefits for biomedicine, drug delivery and diagnostic applications. Courtesy controlled multivalency of dendrimers, a plethora of drug molecules, targeting and solubilizing groups can be linked to their surface. Additionally, due to low dendritic PDI, they exhibit predictable/reproducible clinical pharmacokinetics in contrast to conventional linear polymers. Moreover, unlike dendrimers most traditional linear polymers exhibit uneven coiled structure, however the 3D globular dendritic structure influences biochemical properties, leading to positive outcomes related to their 3D macrostructure. Recently, synthetic or semisynthetic polymers have shown promise in drug delivery as polymeric micelles [16–18], this finding has motivated researchers working on dendrimers to synthesize new macromolecules in their design and development and possible exploration of novel chemotherapeutics.

THE AIM. This article is written aiming to enlighten the readers regarding design, development, limitations, challenges and future directions regarding anticancer bio-dendrimers.

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MATERIALS AND METHODS

The data base was represented by such systems as Medline, Cochrane Central Register of Controlled Trials, Scopus, Web of Science Core Collection, PubMed, Google-Academy. A search was carried out for the following keywords and combinations: Polypropylene imine (PPI); Poly-L-lysine (PLL); Polyamidoamine (PAMAM); cancer; drug delivery; dendrimers.

RESULTS AND DISCUSSION

1. An overview of dendrimers as therapeutic, diagnostic, theranostic and targeted delivery agents

Targeting ligands, drugs and diagnostic agents are attached to dendrimers. Anticancer drug-loaded Drugs bound to dendrimers have been found at higher concentrations in the systemic circulation in addition to enhanced cellular transfection and circumvention of efflux transporter. For instance, At least 50 unbound cisplatin molecules need to be transfected into the cell to exhibit efficacy. Nevertheless, cisplatin-bound dendrimers were found to exhibit better efficacy at lower concentrations of the drug with decreased cytotoxicity.

1.1. Dendrimers in diagnosis of diseases

Apart from acting as potential drug delivery molecules dendrimers have also been explored in diagnostic and imaging applications in cancer treatment [19, 20]. Chemotherapy works either by one or a combination of these ways including angiogenesis inhibition, apoptosis induction, gene expression modulation, signal transduction inhibitor blockage and vaccines. Anticancer drugs can either be enclosed in the core (via hydrogen bonding, hydrophobic interaction or electrostatic attachment) or can be attached covalently to the surface/shell/branches to dendritic end groups [21]. The extent of drug loading depends on the generation of dendrimer being used: higher the generation better is the entrapment, moreover it also offers a plethora of functional groups for drug conjugation.

1.2. Drug release kinetics of dendrimers

The dendrimer-drug interaction governs the fate of drug release from the dendritic complex [22]. The rate of drug release from the core varies significantly from that of dendrimer end groups. Usually the drug bound to the surface releases first and at a faster pace while drug inside core is the last to be release and gives a sustained release effect. Furthermore, the pH and other environmental factors play a key role in drug release. Chemotherapeutic drugs act non selectively and cannot specifically target the tumor, a major challenge in the success of conventional chemotherapy. Henceforth, researchers have devised an approach to selectively target tumor, a strategy similar to antibody-toxin, immunoconjugate concept where potential units/molecules are first identified and then attached to the nanoparticulate/drug delivery system surface which propels the carrier towards the tumor directly without being distributed to all parts of body [23]. An important feature of this approach is to take DNA zipper which allows the targeting agent, e.g. folate targeted PAMAM [24], to be attached to dendritic complex by cDNA. Latest dendritic complexes which possess the capacity to attach carbohydrates are currently using these agents. A good example is the application of dendrimer in vaccine development where oligosaccharides (which are exclusive to cancer cells) are attached onto the dendrimer surface [25–28]. Recently, it has been found that dendrimers direct the carbohydrates' multimeric presentation vital to enhancing the cluster glycoside effect (responsible for enhanced dendrimer targeting) [29–31]. An additional approach to apply glycosylation in cancer treatment involves sialic acid expression on cellular surface by the use of N-acetyl-mannosamine analogs [26, 32]. Dendrimers can load and attach a range of targeting agents which can direct them towards the cancer cells [33, 34]. Biocompatibility and safety of drug delivery systems has always been of concern, however dendrimers have been found to be safe and biocompatible and are easily eliminated out of body. The complexes of drug-PAMAM remain in systemic circulation for long in comparison to unbound free drug. The elimination pathway of dendrimers is renal and they are also used up by growth factors, folic acid, peptides and antibodies [35–39]. In some positively charged dendrimers, peripheral end groups have been found to cause toxicity against normal cells [40–42].

2. Dendrimer types

2.1 Poly(amidoamine) PAMAM dendrimers

Poly(amidoamine) PAMAM are the most commonly used dendrimers in drug deliver due to their hydrophilic, biocompatible and non-immunogenic nature. The cores of PAMAM dendrimers are usually made up of diaminododecane, ethylenediamine, diaminohexane and diaminobutane [43, 44]. The moieties which are used to fabricate branches comprise methyl acrylate and ethylenediamine, possess amine and carboxyl end groups [45].

2.1.1. Anticancer drug loaded PAMAM dendrimers: Doxorubicin (DOX)

One of the most frequently used drug in chemotherapy is doxorubicin. In spite of its numerous efficacious effects it exerts major adverse effects, the most dangerous of which is cardiotoxicity [46]. Many researchers

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around the globe have recently developed dendrimers for drug delivery and they have successfully loaded DOX onto them to reduce its adverse effects thereby increasing its efficacy [47–49]. Zhong et al. [50] while working on pulmonary drug delivery formulated DOX-loaded dendrimers and investigated their activity in decreasing the extent of metastasis when administered locally into lungs. Acid-sensitive hydrazone bonding was used to conjugate DOX to the surface of G4 PAMAM dendrimers. Mice were xenografted with melanoma B16-F10 cells to study the metastasis reducing effect of local pulmonary administration of DOX-loaded dendrimers, the size of tumor was found to be reduced with reduction in cardiac circulation of cancer cells, moreover, the pulmonary accumulation of DOX conjugated dendrimers was also found to be enhanced. The acid-responsive hydrazone bond between the dendrimers and drug helps in stimulus-sensitive release of drug release in tumor or endosomal vesicles [51] on low pH exposure thus enhancing tumor targeting and release.

2.1.2. Anticancer drug loaded PAMAM dendrimers: Paclitaxel (PTX)

In past two decades, researchers have thoroughly studied stimuli responsive drug release and a lot of progress has been made in this area [52,53]. Working on this strategy [54] G4 PAMAM dendrimers were attached to PTX using a peptide linker (which can be cleaved by an enzyme cathepsin B *in vivo*), the PTX-loaded dendritic complex was found to be more cytotoxic to cancer cells (possessing high cathepsin B activity in comparison to normal cells) in contrast to unbound PTX. PTX-loaded dendrimers were found to exhibit better tumor inhibition efficacy than free drug *in vivo* in mice with actively expressing cathepsin B MDA-MB-231 xenograft.

2.1.3. Anticancer drug loaded PAMAM dendrimers: Docetaxel (DTX)

To improve the targeting ability of dendrimers such as PAMAM, their surface can be modified and attached with numerous ligands, this attachment results in offering better tumor targeting with reduced adverse effects [55]. One of the commonly used ligands in active targeting are the antibodies. Kulhari research group [56] used an antibody trastuzumab (TZ) as a ligand and conjugated it to surface of DTX-loaded G4 dendrimers using PEG as linking agent. Human epidermal growth factor receptor type 2 (HER2) are reported to be over expressed in numerous types of cancers, TZ being present on dendrimers surface gets attached to them and stop downstream signaling [57]. Two types of cells such as MDA-MB-453 (HER2 positive) and MDA-MB-231 (HER2 negative) were used to investigate the efficacy and targeting potential of TZ-bearing dendrimers. After a 4 h incubation period, in contrast to DTX-loaded dendrimer (without TZ), 70% higher cellular uptake of TZ-DTX dendrimers was seen in MDA-MB-453 (HER2 positive) cells, whereas no significant difference in cellular uptake was observed in MDA-MB-231 (HER2 negative) cells. Furthermore, in contrast to DTX-loaded dendrimer (without TZ), TZ-DTX dendrimers showed higher cytotoxicity against MDA-MB-453 cancer cells. Additionally, the IC_{50} exhibited by TZ-DTX dendrimers was found to be 3.6-fold greater than the DTX-loaded dendrimer (without TZ), though no considerable difference was observed in the efficacy of any of the formulations or the free drug in MDA-MB-123 cells.

2.1.4. Anticancer drug and siRNA co-loaded PAMAM dendrimers: DOX and siRNA

To address the issue of multidrug resistance (caused by protein P-gp) Pan research group [58] used P-gp analog siMDR-1 in co-delivery of anticancer drug DOX and siRNA and the initial results were promising. PEG-complexed G4 PAMAM dendrimers were co-loaded with siMDR-1 and DOX. PEG helps homogenizing the structure of dendrimers in addition to shielding the cationic charge. PAMAM assists in the complexation of siRNA, enhancing interaction with the cells and aiding in endosomal escape. To enhance the therapeutic potential, maintaining an equilibrium between interaction with cells and cytotoxicity is important. To co-deliver siRNA and DOX, the optimum ratio of MDM was discovered to be 1:10. MDM dendrimers (1:10) complexed with siMDR-1 were found to decrease the function and levels of membrane attached P-gp, hence resulting in decreased multidrug resistance. Together with effectively delivering siRNA to cancer cells and reducing multidrug resistance, the dendrimers also exhibited better cytotoxicity against cancer cells in comparison to free DOX.

2.1.5. PAMAM dendrimers in combination chemotherapy: DOX and Cisplatin

PAMAM dendrimers have been extensively explored in various aspects of drug delivery, an important area is the combination drug delivery. Guo and coworkers [59] studied the effect of loading a combination of chemotherapeutics onto dendrimers. To realize their idea, first they fabricated amine terminated G4 PAMAM dendrimers modified with hyaluronic acid (HA@PAMAM) followed by co-loading (covalent conjugation) of cisplatin and DOX (HA@PAMAM-Pt-Dox). By performing various studies and tests it was found that HA@PAMAM-Pt-Dox dendrimers enhanced the efficacy of cisplatin and DOX against breast cancer, the efficacy of HA@PAMAM-Pt-Dox was found to be better than free/unbound cis-

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platin and DOX combination. Notwithstanding numerous achievements and gains, some challenges were also encountered in this strategy including lack of targeted delivery to cancer cells, drug solubility issues and occasionally issues faced due to drugs' antagonistic nature. The researchers thoroughly studied the physicochemical characteristics of HA@PAMAM-Pt-Dox dendrimers both *in vitro* and *in vivo* and the results positively indicated their synergistic potential in breast cancer therapy.

2.1.6. PH-responsive PAMAM dendrimers surface-decorated with FA in DOX delivery

Working on stimuli-responsive drug release Zhang and coworkers [60] selected partially acetylated G5 PAMAM dendrimers, conjugated folic acid onto the surface followed by DOX conjugation by a pH-sensitive cis-aconityl linkage yielding G5.NHAc-FA-DOX conjugate. FA receptors are known to be overexpressed in a variety of cancers and this is the rationale behind attaching FA onto the surface of drug delivery agents so that they could offer cancer targeting. The fabricated dendrimers co-loaded with DOX and folic acid showed promise in reducing the severity and growth of tumor.

2.1.7. Biotinylated PAMAM dendrimers for Paclitaxel (PTX) delivery

Alongside DOX, researchers have worked on other chemotherapeutics as well to enhance their efficacy and reduce their side effects, Yao and Ma [61] strived to improve cell uptake and reduce unwanted adverse effects of Paclitaxel. In doing so, they performed biotinylation of PAMAM dendrimers and conjugated paclitaxel (PTX) onto them. To assess the level of dendritic biotinylation, 4'-hydroxyazobenzene-2-carboxylic acid (HABA) assay was performed. HABA assay results confirmed a comprehensive dendritic biotinylation. To confirm the retention of the complex's basic integrity, differential scanning calorimetry (DSC) was performed which confirmed the integrity of complex. Following their development, various physicochemical tests including determination of drug loading (%) and *in vitro* drug release were performed to investigate characteristics of the PTX-biotinylated dendrimers complex. To investigate the cell transfection potential of PTX-biotinylated dendrimers in HEK293T and OVCAR-3, a study involving fluorescence was performed. The dendrimer complex exhibited high drug loading 12.09% and a sustained drug release 70% for 72 h in comparison to free drug and other formulations. OVCAR-3 (cancer) cells, in comparison to HEK293T (normal) cells up took more biotinylated dendrimers. Through a set of statistical and experimental studies and experiments it was found that the biotinylated dendrimers release the drug in a sustained manner for up to 72 hours, augmented the cellular uptake with lesser toxicity and adverse effects.

2.1.8. PAMAM dendrimers surface-decorated with Hyaluronic acid (HA) for recombinant methioninase (rMETase) delivery

Li and coworkers [49] strived to deliver chemotherapeutics to gastric cancer (GC), one of the most common causes of cancer-associated deaths. Against GC, recombinant methioninase (rMETase) is commonly used anticancer drug in polymer based nanoparticulate delivery. The researchers developed a novel dendritic drug delivery system comprising G5 PAMAM-Au-METase and surface modified it with Hyaluronic acid (HA), the system exhibited promising biocompatibility, solubility and other characteristics. In an *in vivo* study carried out in Nu/Nu nude mice xenografted with CD44(+) GC cells, HA decorated G5 PAMAM-Au-METase dendrimers were seen to decrease the size of tumor and inhibiting its growth.

2.1.9. PAMAM dendrimers modified with Alkyl PEG and Cholesteryl formate

Pishavar research group [62] modified G5 PAMAM dendrimers into two different ways such as alkyl-PEG and cholesteryl formate modification, additionally they also surface-modified G4 PAMAM with tumor necrosis factor receptor-associated apoptosis-inducing ligand for targeted colon cancer delivery. The resultant modified dendrimers showed better transfection efficiency by overcoming numerous barriers (both extracellular and intracellular) in addition to reducing the toxicity of PAMAM. Furthermore, an *in vivo* study performed in mice bearing C26 tumor xenografts showed the tumor inhibitory potential of the dendritic drug delivery system. An important aspect related to different generations of PAMAM dendrimers is maintaining an equilibrium between the efficacy and toxicity, usually the higher the generation so is the efficacy and toxicity. Considering this factor, many researchers are using G4 PAMAM dendrimers as drug and siRNA/ gene delivery agents owing to their better efficacy and moderate toxicity.

2.2. Poly (propylene imine) PPI dendrimers

After PAMAM, PPI dendrimers are commonly used and they contain a core which is usually made up of 1,4-diaminobutane (DAB), however it can also be synthesized using ethylenediamine or other agents and by double Michael addition. Propylene imine monomers are frequently used as branching units in these dendrimers. Thus, their core is composed of tertiary tris propylene amine monomers, and the surface ends are

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usually made up of primary amines [64]. In contrast to PAMAM, their core is more hydrophobic due to the presence of alkyl chains and amide groups [65].

2.2.1. PPI dendrimers encapsulated with anticancer drug: Melphalan

Kesherwani research group worked on different generations of PPI dendrimers and also modified them [66,67]. G3, G4 and G5 PPI dendrimers were encapsulated with melphalan and G4 and G5 complexes exhibited better inhibition of tumor and prolonged survival in BALB/c mice bearing MCF-7 cell xenografts. As the generation number goes up, so does the hemolytic toxicity of the dendrimers [68]. The targeting ability of these PPI dendrimers was found to be enhanced on FA surface modification, moreover their efficacy was also augmented and toxicity reduced possibly due to cationic group concealment by FA. However, the biocompatibility of G5 was found to be compromised in contrast to lower generations such as G3 and G4. Furthermore, dendrimers surface modified with FA showed better tumor inhibition in BALB/c mice bearing MCF-7 xenografts.

2.2.2. PPI dendrimers encapsulated with PTX and surface decorated with monoclonal antibody

To enhance the targeting efficiency of PPI dendrimers, Jain and coworkers [69] fabricated carboxylic acid-terminated G4.5 PPI dendrimers, surface-decorated them with monoclonal antibody mAbK1 for better targeting and loaded them with chemotherapeutic drug PTX (mAbK1-PPI-PTX). Mesothelin is a protein which has been found to be overexpressed in certain cancers and mAbK1 specifically binds to it. mAbK1-PPI-PTX dendrimers showed better cytotoxicity *in vitro* in OVCAR-3 (mesothelin overexpressed ovarian cancer) cells in comparison to free PTX or PPI-PTX dendrimers. It can be concluded from the findings of numerous physicochemical and *in vitro* experiments that the PTX-loaded G4.5 PPI immune-dendrimers possess potential to efficiently target ovarian cancer cells due to the overexpression of mesothelin receptors on them.

2.2.3. Maltose-modified PPI dendrimers (mal-PPI) surface complexed with siRNA

Tietz research group [70] while working on short interfering RNAs (siRNAs) found their application in cancer treatment. They worked on the development of new polymer nanocarrier built up of transfection disabled maltose-modified PPI dendrimers (mal-PPI) attached to single chain fragment variables (scFvs) for the targeted siRNA delivery. The results showed mal-PPI dendrimers to be efficient carriers of siRNA in cancer therapy, moreover this study also highlighted a novel strategy for bio-conjugation of nano-biomaterials to protein ligands.

2.2.4. PPI dendrimers loaded with anticancer drug: Cytarabine (Ara-C)

Szulc lab [71] worked to improve the already present strategies in leukemia treatment. Cytarabine, abbreviated as Ara-C is a chemotherapeutic drug, notwithstanding its efficacy it faces numerous challenges such as insufficient cellular uptake, buildup in tumor cells rather it should be converted to active triphosphate analogue, and the development of resistance. PPI dendrimers were complexed with nucleotide Ara-C triphosphate (Ara-CTP). PPI glycol-dendrimers efficiently loaded, carried and delivered cytarabine to cancer (1301 and HL-60 leukemia) cells *in vitro*. The results showed potential of the drug-PPI dendritic complex in targeted chemotherapy.

2.2.5. PPI dendrimers surface-decorated with Glycyrrhizin (GL) (GL-PPI) for DOX delivery

et al. [69] developed two different nanocarriers for the delivery of DOX i.e. GL-conjugated PPI dendrimer complex (GL-PPI-DOX) and GL decorated multi-walled carbon nanotubes (GL-MWCNT-DOX) in hepatic cancer. GL-PPI-DOX dendrimers showed better drug loading and entrapment efficiency (87.26±0.57%) in contrast to GL-MWCNT-DOX nanotubes (43.02±0.64%). Moreover, the hemolytic toxicity of DOX was also found to be decreased by 12.38±1.05% in case of GL-PPI-DOX and 7.30±0.63% while loaded onto GL-MWCNT-DOX, and the possible explanation of this phenomenon is the presence of GL in nanocarriers. An *in vitro* (MTT) assay carried out on HepG2 cells exhibited a decrease in the IC_{50} of DOX from 4.19±0.05 μM (of free dox) to 2.7±0.03 in case of GL-MWCNT-DOX and 2.0±0.01 μM for DOX loaded onto GL-PPI-DOX.

2.3. Poly-l-lysine PLL dendrimers

Because of their promising oligonucleotide condensation potential, poly-L-lysine (PLL) dendrimers are frequently employed in siRNA and gene delivery applications [89]. Like other polymers (PAMAM and PPI) used to fabricate dendrimers, PLL also exhibits promising hydrophilic characteristic, elasticity, biocompatibility and biodegradability. The core and the branching monomers are both made up of amino acid lysine, and structural peptide bonds are also prevalent [90]. PLL dendrimers differ from PAMAM and PPI in their asymmetrical nature. However, they still possess specificity with the presence of terminal amine groups and arranged/sequenced number of lysine groups emanating from core. Lysine present in terminal PLL contains two modifiable primary amines that can be functionalized for improved biomedical applications [91, 92].

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Table 1 – Dendrimers in targeted chemotherapy

Dendrimer complex	Cancer type	Payload (Drug/siRNA/gene)	Significant outcomes and findings	Type of Study	Reference
Dendrimers coated with gold nanoparticles	Breast	Curcumin	Reduction in growth and tumor size	*In vitro*	[70]
PAMAM-phosphorous dendrimers		Polo-like kinase 1 siRNA-607	Effective siRNA delivery to tumor interstitium	*In vitro*	[71]
Biotinylated PAMAM-PEG dendrimers	Lung	Paclitaxel	Successful targeted delivery of PTX to biotin receptors	*In vitro*	[72]
Peptide labeled dendrimers		A complex of DNA-Plasmid	Effective gene therapy *in vivo* in RAG1KO mice bearing lung cancer xenografts	*In vitro & In vivo*	[73]
PEG-immobilized, or PEGylated, surfaces and PAMAM dendrimer-immobilized		Glycoprotein-enzyme	Cancer cell detection using enzymes	*In vitro*	[74]
FA-decorated PAMAM dendrimers		cis-diamine platinum and siRNA	Effective receptor-mediated targeted co-delivery of cis-diamine platinum and siRNA	*In vitro*	[75]
Alkyl-modified dendrimers		siRNA	Successful siRNA delivery and gene silencing	*In vitro*	[76]
PAMAM dendrimers	Skin	antisense oligonucleotide	Effective apoptosis in skin cancer	*In vitro & in vivo*	[77]
Phosphorous dendrimer		Methylene blue and rose Bengal	Successful skin cancer therapy	*In vitro*	[78]
Biotinylated-PAMAM dendrimers		cRGD peptide	Successful development of dendrimers for Integrin αVβ3 targeting	*In vitro*	[38]
Akali blue-PAMAM dendrimers	Lymphoma	Paclitaxel	Successful diagnosis and targeted lymphoma therapy	*In vitro*	[39]
PAMAM dendrimers grafted with fatty acid		5-FU	Efficient Lymph absorption and enhanced 5-FU bioavailability	*In vitro & In vivo*	[79]
Lipids-based dendrimers	Ovarian	Lipids-based dendrimers	Successful theranostic applications	*In vitro & In vivo*	[80]
Herceptin- and Diglycolamic acid-functionalized PAMAM dendrimers		Cisplatin	Enhanced ovarian cancer targeting tumor inhibition	*In vitro & In vivo*	[69,81]
PPI immuno-dendrimers		Paclitaxel	Antibody-mediated ovarian cancer targeting and tumor inhibition	*In vitro & In vivo*	[69,82]
PAMAM-peptide dendrimers		Follicle-stimulating hormone receptor (FSHR)	FSH33-mediated ovarian cancer targeted delivery	*In vitro & In vivo*	[69,83]
PAMAM-chitosan dendrimers	Brain	Temozolomide	Successful brain glioblastoma treatment	*In vitro & In vivo*	[84]
Concanavalin-, Sialic acid-, glucosamine-anchored PPI dendrimers		Paclitaxel	Augmented targeted delivery of paclitaxel to brain	*In vitro & In vivo*	[85]
PAMAM dendrimer modified with borneol (Bo) and FA		Doxorubicin	Successful brain glioma delivery	*In vitro & In vivo*	[20, 86]
Anti-EGFR dendrimers		siRNA	Enhanced *in vivo* siRNA brain delivery and gene silencing	*In vitro & In vivo*	[87]
Boronated-PAMAM dendrimer		Cetuximab	Effective neutron capture therapy of glioma	*In vitro & In vivo*	[88]

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2.3.1. PLL dendrimers loaded with anticancer drug: DOX

DOX can be successfully loaded onto PLL dendrimers and its targeted delivery can also be realized resulting in better chemotherapeutic activity and less adverse effects [66, 92]. G6 PLL dendrimers (without carrying any drug) with strong cationic charge showed efficient *in vivo* anticancer activity in mice bearing B16F10 xenografts [93]. Another study found these dendrimers to exhibit deep *in vivo* penetration in mice bearing B16F10 melanoma xenografts and *in vitro* 3D DU145 prostate cancer tumor model, accrediting to their small average diameter and strong cationic charge [94, 95]. Li research group [96] also strived to improve DOX targeted delivery by using G6 PLL dendrimers. Niidome et al. [97] found higher PLL dendrimers tumor accumulation and reduction in tumor size *in vivo* in BALB/cN mice bearing Colon-26 mouse rectum carcinoma xenografts, with apparently no adverse effects. The attachment of PEG to PLL dendrimers resulted in improved accumulation in tumor by enhanced permeation and retention (EPR) effect, while the presence of oligopeptide bond created a hydrophobic cavity leading to enhanced DOX encapsulation. Some PLL dendrimers are in Phase I clinical trials such as a PEGylated-PLL dendritic delivery system surface-modified with docetaxel DEP® (Starpharma, Australia) exhibited improved targeted delivery and tumor (breast, ovarian, lung and prostate) inhibition efficiency than that by Taxotere® (docetaxel), an established anticancer drug [88]. Jain lab also studied the chemotherapeutic potential of PLL dendrimers in the treatment of cancer. The researchers developed PLL dendritic system surface decorated with FA (FPLL) as a DOX nanocarrier to enhance antiangiogenesis, tumor cell cytotoxity, targeted DOX delivery and a pH-responsive release. Ryan et al. [98] developed and compared the *in vivo* anti-lymphoma activities of three different drug delivery systems including PEGylated-PLL dendrimers loaded with DOX, DOX-loaded PEGylated liposomes and DOX-encapsulated pluronic micelles by studying their plasma and lymph pharmacokinetics. The results revealed that on subcutaneous and intravenous dosing the PEGylated-PLL dendrimers substantially augmented the recovery of DOX in thoracic lymph better than the DOX-encapsulated pluronic micelles.

2.3.2. PLL dendrimers surface-complexed with siRNA

PLL possesses potential to efficiently attach and condense siRNA/gene on to its surface, a characteristic courtesy which it has gained a lot of attention by researchers. Patil and coworkers developed a triblock PAMAM-PEG-PLL dendritic system for targeted siRNA delivery and gene silencing. Each monomer of the triblock was carefully selected and had certain roles to play for instance PAMAM acted as a proton sponge and aided in endosomal escape and the cytoplasmic delivery of siRNA; likewise PEG linked PLL to PAMAM, provided stability against nucleases and also helped retain siRNA integrity in plasma; moreover, PLL provided enhanced transfection and penetration, and strong siRNA binding onto the surface by the presence of primary amines. Apparently no toxicity related to the triblock polymer was reported, moreover the toxicity of PLL was also found to be significantly reduced, and the possible explanation to this observation is PEG-PAMAM conjugation. The findings revealed promising transfection efficiency of the triblock PAMAM-PEG-PLL dendritic system into cancer cells and also exhibited significant stability in plasma.

CONCLUSION

Dendrimers have seen considerable growth and progress in their design and development for biomedical applications during last two decades. Dendrimers, due to their globular structure and polyvalent character possess potential to address the challenges and problems faced by conventional drug delivery such as poor solubility, non-selective delivery and poor bioavailability and distribution. Moreover, dendrimers have also recently shown promise in the areas of imaging; diagnostics, theranostics, targeting drug delivery and others.

An area that still needs an in depth analysis and attention of researchers is acquiring more information regarding the bioavailability and distribution of dendrimers so that these characteristics could be optimized for best effect. Dendrimers when administered *in vivo* should be able to stay in plasma long enough to gather at the target sites, nonetheless their timely elimination out of the body is also equally important to avoid causing toxicity or other adverse effects, these areas need further attention and research. Another major challenge is to predict the fate of dendrimers (tissue localization) *in vivo* in advance; additionally, the effect of peripheral groups on the physicochemical properties of dendrimers also needs to be studied in depth. Drug release and kinetics is another field which needs more attention of researchers and can be significantly improved in getting more predictable and reproducible. The alteration/modification of enzymatically cleavable links in dendrimers is challenging due to compressed 3D globular dendrimer structure; nonetheless, dendrimers are useful platforms for using alternate release pathways such as cascade release. Few researchers have reported their findings lately in this area; however, more studies are needed to draw a conclusion.

The unique characteristics, qualities and advantages of large dendrimers over other linear polymers lie behind their stepwise synthesis; newest discoveries have shown that the dendritic nanocarriers have many unique features that endorse more research and development.

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BIOLOGICAL ACTIVITY OF *HYPERICUM PERFORATUM* L. (HYPERICACEAE): A REVIEW

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Hypericum perforatum L. (St. John's wort) is a medicinal plant that has been intensively studied by clinicians, pharmacologists, and chemists. It has resulted in the publication of both original articles and a number of reviews devoted to the general spectrum of the biological activity of its extracts and the separate chemical components of this species. Unlike many other known medicinal plants, the pharmacological study of which is accompanied by the establishment of new (or rediscovered) structures of chemical compounds, the dynamics of the present study of *H. perforatum* is mostly associated with a detailed study of the mechanisms of its therapeutic effect and less with the search for new components.

The aim of this work is to review and analyze the data on the biological activity of extracts and individual compounds of *Hypericum perforatum* L. (Hypericaceae), or St. John's wort, published in the scientific literature over the past 10 years.

Materials and methods. To collect and analyze the information, such electronic databases as PubMed, Scopus, Web of Science, Google Scholar, and other available resources have been used. The following keywords and word combinations were used for search in the databases for 2010–2020: "*Hypericum perforatum*", "St. John's wort", "the biological activity of St. John's wort", "hypericin", "hyperforin".

Results. The review provides information on antidepressant, neuroprotective, nootropic, anxiolytic activity, antibacterial, cytotoxic, anti-inflammatory properties, analgesic, hypoglycaemic effects, and other types of activity of *H. perforatum* extracts, as well as individual compounds (hypericin, hyperforin, amentoflavone, and others) isolated from this species. It is well known that the secondary metabolites of St. John's wort are naphthodianthrons, flavonoids and other phenolic compounds, several classes of lipophilic substances including phloroglucinol derivatives and terpenoids. Apart from extracts and their fractions, the biological activity of photoreactive naphthodianthrone hypericin and hyperforin (a phloroglucinol derivative) has been studied in detail.

This review provides an analysis of published data from 2010 to 2020 on the biological activity of St. John's wort. At the present time *H. perforatum* is primarily well-known for its antidepressant-like properties, which are confirmed by numerous pharmacological studies and clinical trials. Still there is no consensus on the effective treatment of severe or even moderate depression with St. John's wort. This review also provides information on the neuroprotective, nootropic, antiepileptic, anxiolytic, antimicrobial, antiviral, antiprotozoal, antitumor, cytotoxic, analgesic, anti-inflammatory and other effects of *H. perforatum* extracts, as well as its individual compounds.

Conclusion. Despite the popularity of *H. perforatum* as a plant with an antidepressant-like activity, intensive research work continues to be carried out to elucidate the molecular mechanisms of the actions of extracts and individual compounds in disorders of the nervous system. Studying its antibacterial, antiviral, and cytotoxic activity may also open up some great prospects, along with determining the possibility of using St. John's wort in metabolic disorders, genitourinary disorders, and other fields of medicine.

Keywords: St. John's wort; antidepressant; neuroprotective; nootropic; anxiolytic; antibacterial; cytotoxic; hypoglycaemic activity; hypericin; hyperforin; amentoflavone

Abbreviations: ROI – reactive oxygen intermediate; GABA – γ-aminobutyric acid; K562 – K-lines of acute erythroid leucosis; MAO-A – monoaminooxidase A; cAMP – cyclic adenosine monophosphate; CNS – central nervous system; A375 – human melanoma cell line; A375, 501mel – unpigmented melanoma cell lines; ADAMTS8, ADAMTS9 – a disintegrin-like and metalloprotease with thrombospondin type 1 motif 8, 9; BDNF – brain-derived neurotrophic factor; CaMK-IV – calcium/calmodulin-dependent protein kinase; cAMP – cyclic adenosine monophosphate; CLL – chronic lymphocytic leukemia cell line; COX – cyclooxygenases; CREB – cAMP response element-binding protein; CUMS – chronic unpredictable mild stress; CXCL9, CXCL10,

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– C-X-C motif chemokine; CYP3a CYP2c – cytochromes; D273 – medulloblastoma cell line; GABA – γ-aminobutyric acid; HT-29 – colon adenocarcinoma cell line; HT22 – immortalised mouse hippocampal neuronal cell line; iNOS – inducible nitric oxide synthase; JAK1 – janus kinase 1; JEG-3 – choriocarcinoma cell line; K562 – acute erythroid leukemia cell line; MAO-A – monoamine oxidase A; MAPK – mitogen-activated protein kinase; MCF-7 – human breast cancer cell line; MEK – mitogen-activated protein kinase kinase; MG-63 osteosarcoma cell line; NGF – nerve growth factor; NMDAR – N-methyl-D-aspartate receptor; PC12 – pheochromocytoma cell line; PGE_2 – prostaglandin E2; PI3K – phosphatidylinositol 3-kinase; PKB/Akt – protein kinase; RINm5F – insulinoma cell line; SCC – human squamous carcinoma cell line; SH-SY5Y – neuroblastoma cell line; TNFα – tumor necrosis factor α; TrkB – tropomyosin-related kinase B; TRPM2, TRPV1, TRPC6 – transient receptor potential cation channel; U937 – human acute myeloid leukemia cell line; UCT Mel-1 – pigmented melanoma cell line; β_1-AR – β_1-adrenergic receptors.

БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ *HYPERICUM PERFORATUM* L. (HYPERICACEAE): ОБЗОР

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Hypericum perforatum L. (зверобой продырявленный) является лекарственным растением, которое в последнее время интенсивно изучается клиницистами, фармакологами и химиками. Результатами этого являются публикации как оригинальных статей, так и ряда обзоров, посвященных спектру биологической активности экстрактов и отдельных химических компонентов этого вида. В отличие от многих других известных лекарственных растений, фармакологическое изучение которых сопровождается установлением структур новых (или вновь обнаруженных) химических соединений, динамика современного изучения *H. perforatum* по большей части связана с детальным изучением механизмов его терапевтического действия и, в меньшей степени, с поиском новых компонентов.

Цель: обзор сведений по биологической активности экстрактов и отдельных компонентов *Hypericum perforatum* L. (Hypericaceae) – зверобоя продырявленного, опубликованных в научной литературе за последние 10 лет.

Материалы и методы. Для сбора и анализа сведений использовали электронные базы данных PubMed, Scopus, Web of Science, Google Scholar и др. доступные ресурсы. Поиск в базах данных производился по публикациям за 2010–2020 гг. по таким ключевым словам, как: *Hypericum perforatum*, зверобой продырявленный, St. John's wort, биологическая активность зверобоя.

Результаты. В обзоре представлены сведения об антидепрессивной, нейропротекторной, ноотропной, анксиолитической активности, антибактериальным, цитотоксическим, противовоспалительным свойствам, анальгезирующем, гипогликемическом действии, а также других видах активности экстрактов *H. perforatum* и индивидуальных соединений (гиперицина, гиперфорина, аментофлавона и др.), выделенных из этого вида. Как известно, пул вторичных метаболитов этого вида включает нафтодиантроны, флавоноиды и другие фенольные соединения, несколько классов липофильных веществ, в том числе производных флороглюцина и терпеноиды. При этом наиболее подробно (помимо экстрактов и их фракций) изучалась биологическая активность фотореактивного нафтодиантрона гиперицина и гиперфорина – производного флороглюцина. Данный обзор посвящен анализу сведений по биологической активности зверобоя продырявленного, опубликованных в литературе с 2010 по 2020 годы. В настоящее время популярность *H. perforatum* связана прежде всего с его антидепрессивными свойствами, которые подтверждены многочисленными доклиническими исследованиями и клиническими испытаниями, хотя до сих пор нет единого мнения о возможности эффективности использования зверобоя для лечения как тяжелой, так и даже умеренной депрессии. Кроме того, в данном обзоре приведены сведения о нейропротекторной, ноотропной, противоэпилептической, анксиолитической, антибактериальной, антивирусной, противопротозойной активности, противоопухолевых, цитотоксических, анальгезирующих, противовоспалительных и других свойств экстрактов и индивидуальных компонентов этого вида.

Заключение. Несмотря на известность *H. perforatum,* зверобоя продырявленного, как растения с антидепрессивной активностью, продолжаются интенсивные исследования, направленные на выяснение молекулярных механизмов действия экстрактов и индивидуальных соединений при патологиях нервной системы. Кроме этого, весьма перспек-

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тивными могут стать исследования его антибактериальной, антивирусной, цитотоксической активности, наряду с определением возможности применения з. продырявленного при нарушениях обмена веществ, функций мочеполовой системы и в других областях медицины.
Ключевые слова: зверобой продырявленный; *H. perforatum*; антидепрессивная активность; нейропротекторная активность; ноотропная активность; анксиолитическая активность; антибактериальная активность; цитотоксическая активность; гипогликемическая активность; гиперицин; гиперфорин; аментофлавон
Список сокращений: АФК – активные формы кислорода; ГАМК – γ-аминомасляная кислота; К562 – клетки линии К562 острого эритроидного лейкоза; МАО-А – моноаминооксидаза А; цАМФ – циклический аденозинмонофосфат; ЦНС – центральная нервная система; А375 и 501mel – клеточные линии непигментированных клеток меланомы А375 и 501mel; ADAMTS8 – дезинтегрин и металлопротеиназа с мотивом тромбоспондина 8; ADAMTS9 дезинтегрин и металлопротеиназа с мотивом тромбоспондина 9; BDNF – мозговой нейротрофический фактор; CaMK-IV – Ca^{2+}/кальмодулин-зависимая киназа IV типа; CLL – клетки линии CLL хронической лимфоцитарной лейкемии; COX2 – циклооксигеназа 2; CREB – фактор транскрипции CREB; D273 – клетки линии D273 медуллобластомы; HT-29 – клеточния линия HT-29 аденокарциномы толстой кишки; iNOS – индуцируемая NO-синтаза; JAK1 – янус-киназа 1; MAPK – митоген-активируемая протеинкиназа; MCF-7 – клетки линии MCF-7 рака молочной железы; MEK – киназа митоген-активируемой протеинкиназы; mPGES – микросомальная простагландинсинтаза; NMDA – N-метил-D-аспартатные рецепторы; PC12 – клетки феохромоцитомы PC12 ; PI3K – фосфатидилинозитол-3-киназа; PKB – протеинкиназа B; RINm5F – клетки линии RINm5F инсулиномы; SCC – клетки линии SCC чешуйчатой карциномы человека; TNFα – фактор некроза опухоли α; TrkB – тропомиозиновый тирозинкиназный рецептор B; TRPM2, TRPV1, TRPC6 – каналы транзиторного рецепторного потенциального катиона TRPM2, TRPV1, TRPC6; U937 – клетки линии U937 острой миелоидной лейкемии; UCT Mel-1 – клеточная линия UCT Mel-1 пигментированных клеток меланомы; β_1-АР – β_1-адренорецептор

INTRODUCTION

Despite the fact that *Hypericum perforatum* L. (St John's wort) has been known for its medicinal properties for more than 2000 years, it has not yet lost its popularity and continues to be studied intensively by clinicians, pharmacologists, and chemists. The indicator of the active interest in *H. perforatum* is the number of reviews published over the last decade. They are devoted to both the general biological activity profile of its extracts and individual chemical components, as well as to specific types of activity, which are considered in the corresponding sections of this article.

Unlike many other medicinal plants, pharmacological studies of which are accompanied by the determination of the structures of certain new (or rediscovered) chemical compounds, modern research of *H. perforatum* is more focused on the mechanisms of its therapeutic action, and less on the identification of new components.

It is well known that among the secondary metabolites of St. John's wort, there are naphthodianthrons, flavonoids and other phenolic compounds, several classes of lipophilic substances including phloroglucinol derivatives and terpenoids. Apart from extracts and their fractions, the biological activity of hypericin, a photoreactive naphthodianthrone, and hyperforin — a phloroglucinol derivative, have been studied in most detail [1–5].

Today, *H. perforatum* is known primarily for its antidepressant-like properties, which have been confirmed by numerous preclinical studies and clinical trials. Still, there is no consensus on the effectiveness of St. John's wort for severe or at least moderate depression (e.g. [2]).

And yet, the aforementioned beneficial properties of *H. perforatum*, as well as those yet unknown, continue to be studied with unceasing regularity, in different models and within different approaches. A brief (but by no means exhaustive) summary of such studies conducted over the past decade, is presented in this review.

THE AIM of this work is to review and analyze the data on the biological activity of extracts and individual components of *Hypericum perforatum* L. (Hypericaceae), or St. John's wort, published in the scientific literature over the past 10 years.

MATERIALS AND METHODS

To collect and analyze the information, electronic databases PubMed, Scopus, Web of Science, Google Scholar, and other available resources have been used. The following keywords and word combinations were used for search in the databases for 2010-2020: "*Hypericum perforatum*", "St. John's wort", "the biological activity of St. John's wort", "hypericin", "hyperforin".

RESULTS AND DISCUSSION

Antidepressant activity

Despite St. John's wort being a popular "mild" treatment choice for depression, its mechanism of action is not entirely known yet. According to current understanding, among its most active components are the naphthodianthrone hypericin, the phloroglucinol derivatives hyperforin and adhyperforin, the biflavonoid amentoflavone, and other flavonoids [6–13].

According to a systematic review and a meta-analysis, which included 27 clinical trials, St. John's wort is as effective in the patients with mild-to-moderate depression as are some of the most common synthetic antidepressants, at the same time being better tolerated [14–16]. *H. perforatum* is most effective in patients with mild and moderate depression [15–18], and in those experiencing pronounced somatization and gastrointestinal symptoms [19].

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Standardized *H. perforatum* extracts WS 5572, LI 160, WS 5570, ZE 117 were found to be as effective for mild depression as sertraline and imipramine [20, 21]. In moderate depression, *H. perforatum* did not differ in effectiveness from citalopram [22] while surpassing paroxetine [23]. According to a retrospective observational study, IperiPlex®, a multi-fractionated *H. perforatum* extract, was significantly more effective in the patients with moderate depression than Nervaxon®, a mono-fractionated extract [24]. However, *H. perforatum* effectiveness in patients with moderate and severe depression remains somewhat unclear [25, 26].

A multicenter observational study showed the drugs Helarium® and Helarium-425®, both containing extracts of *H. perforatum,* to be well-tolerated by patients with mild to moderate depression [27]. However, *H. perforatum* treatment was associated with a greater frequency of some specific adverse events, including damage to the nervous system, reproductive organs, eyes, ears, liver, and kidneys [28]. A case of psychosis has been reported in a patient who self-administered *H. perforatum* as a herbal infusion [29].

H. perforatum extract potentiated yohimbine toxicity, and exhibited maximal antidepressive activity at 90 mg/kg [14]. Administered over time, both *H. perforatum* ethanolic extract and fluoxetine were found to affect hippocampal and hypothalamic gene expression in chronically stressed rats. Among those affected were genes coding for a number of biomolecules involved in neuroinflammatory and oxidative stress pathways, and some of those associated with Alzheimer's disease [30].

In 2018, T. Herraiz et al. showed that in various dosage forms, *H. perforatum* inhibited monoamine oxidase A (MAO-A). Of all the identified plant constituents, quercetin (IC_{50}= 3.4 μg/ml) and its glycosides were found to be the most active; hypericin (IC_{50} = 17.9 μg/ml) did not contribute significantly to the overall effect of the preparations, and hyperforin failed to show any activity throughout the studied concentration range. According to the authors, taking into account the average content of active ingredients in total preparations with *H. perforatum*, the observed MAO-A inhibition was most likely an additive effect [31].

When compared to venlafaxine, a serotonin and norepinephrine reuptake inhibitor, hypericin decreased blood corticosterone levels, prevented weight loss and anorexia, reduced anhedonia, and stimulated exploratory behaviour in rats with a chronic unpredictable mild stress (CUMS). Moreover, those effects had a shorter onset in the hypericin-treated group than in the venlafaxine-treated one. At the same time, hypericin affected the metabolism of norepinephrine, serotonin, and excitatory amino acids (glutamate and glutamine) [12]. Hypericin also inhibited calcium ion influx into hippocampal neurons, thus increasing an action potential duration, which could possibly play a role in enhancing a synaptic efficiency [32].

Pretreatment of glioblastoma cells with hyperforin and hyperoside hindered lateral mobility of β_1-adrenergic receptors (β_1-AR) and caused their internalization. The two compounds reduced cyclic adenosine monophosphate (cAMP) formation by 10% and 15%, respectively, and by 23% and 15% under subsequent cell stimulation with 5 μM dobutamine. Similar effects were observed when cells were pretreated with desipramine, a tricyclic antidepressant [7]. *H. perforatum* extract and hyperforin were found to increase presynaptic calcium concentrations and thus stimulate the release of the excitatory neurotransmitter glutamate [33].

B. Pochwat et al. found that hyperforin potentiated antidepressant-like activity of lanicemine, a N-methyl-D-aspartate receptor (NMDAR) antagonist, in chronic corticosterone-treated mice as well as in healthy controls. A combination of lanicemine and hyperforin increased the expression of synapsin I, a subunit of glutamate receptor A_1, and neurotrophin BDNF (brain-derived neurotrophic factor) in frontal cortical neurons. Hyperforin also attenuated cognitive dysfunction caused by dizocilpine, a NMDAR antagonist having marked dissociative and psychedelic properties. Nonetheless, 0.3–10 μm hyperforin did not affect NMDAR electrical activity *in vitro* [13].

Much less is known about adhyperforin than about its parent compound, hyperforin. However, adhyperforin has also been shown to exert an antidepressant-like activity, stimulate exploratory behaviour, and reduce anhedonia and hypodynamia in rodents. It suppressed norepinephrine, serotonin and dopamine reuptake *in vitro*, and, just like hyperforin, antagonized reserpine-induced effects *in vivo* [8, 34].

Neuroprotective activities

An ethanolic extract of *H. perforatum* containing 6.0% of hyperforin stimulated neurite outgrowth in HT22 hippocampal cells, increased their resistance to glutamate toxicity, and inhibited the release of tumor necrosis factor α (TNFα) from macrophages [35].

Pretreatment of PC12 pheochromocytoma cells with an *H. perforatum* extract increased their viability at toxic concentrations of hydrogen peroxide, and prevented DNA fragmentation [36]. Another *H. perforatum* extract normalized lateral mobility of integral membrane proteins and phospholipids in glioblastoma cells, which made a more efficient transmembrane signal transduction possible [37]. Ethyl acetate, water, and methanolic extracts of *H. perforatum* inhibited acetyl- and butyrylcholinesterase; ethyl acetate and water extracts also inhibited tyrosinase [38].

An extract of *H. perforatum* reduced the severity of oxidative stress in leukocytes obtained from patients with multiple sclerosis. Cell apoptosis was largely prevented through the activation of antioxidant systems and normalization of intracellular calcium levels [39]. A similar effect associated with the blockade of calcium channels by *H. perforatum* was also observed in rat

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dorsal root ganglion neurons [40]. Later, some of the active constituents of *H. perforatum* were found to inhibit TRPM2 and TRPV1 channels, which mediate calcium ion influx under oxidative stress conditions [41].

A hyperforin-enriched (6.0%) extract (4 mg/kg/d×45 d) effectively prevented degeneration of nigral neurons induced in rats by a chronic exposure to rotenone. Quite on the contrary, an extract containing only 0.2% hyperforin and pure quercetin both exhibited significantly lower activity when administered by the same route and at the same doses [42]. The rats treated with an ethanolic extract of *H. perforatum* (200 mg/kg/d) for 1 week before and 1 week after the administration of 6-hydroxydopamine showed an increase in survival rates of nigral neurons, as well as an attenuation of astrogliosis, inflammation, oxidative stress, and motor dysfunction as compared to control animals [43]. Both St. John's wort extract and pure hyperforin alleviated the symptoms of experimental autoimmune encephalomyelitis, a common model of multiple sclerosis, in mice [44, 45]. An ethanolic extract of St. John's wort also prevented apoptosis of neurons and attenuated oxaliplatin-induced neurotoxicity in rats [46].

In the study by S. Valvassori et al., an *H. perforatum* extract (300 mg/kg /d × 28 d) significantly impaired memory acquisition and object recognition, and decreased the levels of the transcription factors BDNF and NGF (nerve growth factor) in rat hippocampi [47].

In an *ex vivo* experiment in isolated hippocampal neurons, hyperforin (0.3 μM, 24 h) promoted the formation of stubby dendritic spines and, at the same time, decreased the proportion of thin spines [48]. Interestingly, similar alterations in spine morphology have been observed for classical antidepressant agents such as fluoxetine [49], imipramine, and rolipram [50], but in those cases, they had a significantly slower onset, and most probably differed in nature [48].

Hyperforin activated MEK (mitogen-activated protein kinase), MAPK (mitogen-activated protein kinase), phosphatidylinositol 3-kinase (PI3K), protein kinase B (PKB/Akt), and calcium/calmodulin-dependent protein kinase (CaMK-IV) in PC-12 pheochromocytoma cells and hippocampal neurons [51]. These changes culminated in the phosphorylation and activation of the transcription factor CREB (cAMP response element-binding protein), which is considered to be a promising therapeutic target for the treatment of Alzheimer's disease [52–54]. Moreover, the therapeutic effect of *H. perforatum* in Alzheimer's disease was seemingly independent of hyperforin concentration in the preparation [55]. Hyperforin was confirmed to exert neuroprotective effects against aluminum maltolate-induced toxicity in PC12 and SH-SY5Y cells [56].

Hyperforin stimulated CREB phosphorylation, induced TRPC6 calcium channel and TrkB BDNF receptor expression in embryonic mouse cortical neurons [57]. The extract of *H. perforatum* reduced a β-amyloid accumulation and increased levels of P-glycoprotein in the brain tissue of transgenic mice with Alzheimer's disease. However, another study found an *H. perforatum* extract, hyperforin, and high concentrations of quercetin to inhibit P-glycoprotein activity in brain capillary endothelial cells [59]. A methanolic extract of *H. perforatum* inhibited acetylcholinesterase and reduced glutamate levels, at the same time potentiating noradrenergic and dopaminergic neurotransmission in an aluminum chloride-induced rat model of Alzheimer's disease. The extract caused a decrease in β-amyloid deposition rates and ameliorated oxidative stress in treatment groups [60]. A 28 days-long *H. perforatum* treatment course inhibited neuroinflammation, lipid peroxidation, and lowered blood proinflammatory cytokines levels in rats subjected to mechanic sciatic nerve injury [61].

An intracerebroventricular administration of hyperforin to rats subjected to middle cerebral artery occlusion, significantly reduced infarct volume and post-stroke neurological deficit. Hyperforin inhibited the calpain-mediated TRPC6 channel degradation, thus maintaining normal CREB activity and, ultimately, increasing neuron viability following ischemia [62].

TRPC6 activation is thought to be a non-essential or, at the very least, not the only mechanism of a hyperforin action [9, 34]. For instance, a complete absence of TRPC6 had no effect on inward membrane ion currents in microglial cells treated with a hyperforin solution. Its molecule being highly lipophilic, and its properties depending heavily on pH of the medium, it was suggested that hyperforin acted as a protonophore and induced a transmembrane proton transfer in a channel-independent fashion [34]. However, in an *in vivo* experiment in mice, its neurotropic activity was blocked completely by a prior administration of either larixyl acetate or MK 2206, which inhibited TRPC6 and PKB, respectively [13].

Amentoflavone and hypericin are thought to have an opposite effect on a MAPK pathway activity compared to hyperforin [63–65]. Amentoflavone protected HT22 hippocampal neurons against glutamate-induced excitotoxic injury. Besides maintaining the activity of some of the most important antioxidant enzymes and inhibiting reactive oxygen species generation, it inhibited MAPK phosphorylation [66].

Amentoflavone has been shown to exert a direct effect on cholinergic neurotransmission in the central nervous system. It significantly attenuated scopolamine-induced retrograde amnesia through inhibition of acetylcholinesterase and enhancement of antioxidant enzymes activity, thus perpetuating long-term spatial memory [67].

Nootropic activity

A 2016 meta-analysis confirmed *H. perforatum* to possess a significant nootropic activity independent of its antidepressant-like activity. The authors suggested

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that the modulation of 5-HT_2 serotonin receptor activity, dopaminergic, glutamatergic, and γ-aminobutyric acid (GABA)-mediated neurotransmission was among the possible mechanisms of *H. perforatum* nootropic action [68]. Long-term *H. perforatum* treatment has been shown to inhibit the release of adrenocorticotropin and, as a result, that of the glucocorticoid corticosterone, which is the main hormonal mediator of chronic stress response in rodents [68, 69]. An *H. perforatum* extract (125, 250 or 500 mg/kg/d × 30 d) prevented an increase in corticosterone and TNF-α levels in blood and hippocampus in bilateral ovariectomized rats [70].

The nootropic effects of *H. perforatum* preparations have been confirmed experimentally using acute [71] and chronic restraint stress models [72], and a model of cognitive deficit associated with diabetes mellitus [73].

H. perforatum preparations have been demonstrated to have a beneficial effect on neuronal synaptic plasticity in animal [74, 75] and human studies [76]. A single dose of 250 mg of *H. perforatum* tabletted dry extract (Remotiv®) improved short-term verbal and spatial memory in healthy volunteers. Quite surprisingly, no nootropic effect was observed at the dose of 500 mg, although both doses improved the patients' mood and emotional stability. Similarities to and differences from some other neurotropic agents such as citalopram, bromocriptine, and sulpiride, as well as the inverse dose-dependency of *H. perforatum* effects suggest that its primary mechanism of action could involve the augmentation of dopaminergic transmission [77].

The effectiveness of *H. perforatum* for the treatment of autism spectrum disorder is limited. St. John's wort modestly improved irritability, stereotypy and abnormal speech patterns, while clinician ratings on several symptom assessment scales remained unchanged [78].

Antiepileptic activity

Amentoflavone exhibited antiepileptic properties in a number of *in vitro* and *in vivo* studies. It attenuated oxidative stress, inhibited neuroinflammation, and increased GABA binding affinity to $GABA_A$ receptors [79–81]. An ether extract of *H. perforatum* lowered the seizure threshold and increased the after-discharge duration, while *n*-butanolic and water extracts, on the contrary, inhibited epileptogenesis [82]. A methanolic extract of *H. perforatum* reduced seizure duration and mortality in a mouse model of picrotoxin-induced epilepsy [83].

Anxiolytic activity

Anxiolytic properties of *H. perforatum* are related to its nootropic, neuroprotective and antidepressant-like kinds of activity, and are thought to be mediated by its effects on monoaminergic transmission and neuroinflammatory processes [71, 74].

The anxiolytic effect of amentoflavone (25 mg/kg), observed in mice following a single administration, was reduced by pretreatment with flumazenil, a benzodiazepine receptor antagonist. This fact suggested that amentoflavone exerted its anxiolytic effect through the interaction with the benzodiazepine-binding site of the $GABA_A$ receptor [84]. This mechanism of action was subsequently confirmed by radioligand binding assays [85].

Crupi et al. revealed that three-week treatment with an *H. perforatum* methanolic extract decreased anxiety in mice with chronic corticosterone-induced stress [74]. Extract-treated (50 or 100 mg/kg/d × 5 d) mice exhibited higher levels of exploratory activity and were less anxious following six hours of acute restraint stress, although those parameters still fell outside normal ranges [71]. An *H. perforatum* extract (100 or 200 mg/kg/d × 14 d) ameliorated anxiety and depression in streptozotocin-induced type II diabetic rats [86].

Antimicrobial, antiviral and antiprotozoal activity

Antibacterial activity of *H. perforatum* has been reviewed by Z. Saddiqe et al. in 2010 [87]. The antibacterial activity of individual compounds and extracts of St. John's wort is somewhat unclear. Aerial parts macerated with olive oil showed little overall activity, and only a few samples were active against *Trypanosoma brucei rhodesiense* and *Staphylococcus aureus* [88]; hyperforin (but not hypericin) also moderately inhibited *Staphylococcus aureus* growth [89]. The aqueous fraction of an ethanolic extract of St. John's wort suppressed *Streptococcus sobrinus* and *Lactobacillus plantarum* growth [90], and an alcoholic extract and hypericin were active against *Lactobacillus acidophilus*, allowing to consider them as potential oral disinfectants [91]. Photoactivated hypericin inhibited *Candida albicans*, *C. parapsilosis, C. krusei* [92], and *Staphylococcus aureus* growth, but did not affect that of *Escherichia coli* [93]. Hyperforin and a methanolic extract of the aerial parts of the plant were active against *Mycobacterium JLS*, although hypericin and pseudohypericin were not [94]. An ethyl acetate extract of *H. perforatum* exhibited antiviral activity against infectious bronchitis virus *in vitro* and *in vivo* (IBV strain M41) [95], human influenza virus A/PR/8/34 H1N1 [96], influenza A virus [97], and hepatitis B virus [98].

Antitumor and cytotoxic properties

An overview summarizing existing knowledge on the anticancer activity of *Hypericum* species was published in 2017 [99]. It was established that ultraviolet radiation increased the antiproliferative activity of a water/alcohol extract in human melanoma A375 cell line [100]. Investigations are underway to assess the cytotoxic activity of *H. perforatum* components in photodynamic therapy. For instance, hyperforin and aristofolin (a synthetic derivative of hyperforin) induced apoptosis of HT-29 colon adenocarcinoma cells subjected to hypericin-mediated photodynamic therapy [101], and death of both unpigmented (A375 and 501mel) and pigmented (UCT

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Mel-1) melanoma cells [102][1]. Photoactivated hypericin decreased the viability of RINm5F insulinoma cells, human squamous carcinoma cells (SCC) [104], D273 medulloblastoma cells [105], and was effective against anaplastic thyroid cancer [106]. A flower extract inhibited growth and induced apoptosis of K562 (acute erythroid leukemia) cells [107], an ethanolic extract blocked proliferation and induced apoptosis of MCF-7 human breast cancer cells [108], and hyperforin induced death of CLL (chronic lymphocytic leukemia) cells *ex vivo* [109]. Hypericin exerted a cytotoxic effect on MCF-7 cells [110], promoted expression of genes coding for metalloproteinase family enzymes ADAMTS9 and ADAMTS8 having anti-angiogenic and antitumor properties in MCF-7 cells [111]. Hyperforin induced apoptosis of U937 (human acute myeloid leukemia) cells line [112]. An essential oil showed anti-angiogenic properties [113].

Analgesic, anti-inflammatory and wound-healing properties

There has been published a number of reviews focused on the anti-inflammatory and analgesic properties of *H. perforatum* extracts and their components [114–118]. A dry extract relieved neuropathic pain in an experimental study [119]. Hypericin inhibited the pro-inflammatory enzyme janus kinase 1 (JAK1) *in silico,* which could explain its anti-inflammatory properties [120].

Hyperforin inhibited the activity of cyclooxygenases (COX) 1 and 2 and microsomal prostaglandin synthase PGE_2, which play key roles in inflammation and tumorigenesis [121]. Over the past decade, the investigations of anti-inflammatory properties of the four-component fraction of *H. perforatum* ethanolic extract containing amentoflavone, quercetin, chlorogenic acid and pseudohypericin, have been continued [122, 123]. The extract was found to be devoid of anti-inflammatory activity, in contrast with the four compounds [124]. *H. perforatum* flowering tops extract suppressed the expression of proinflammatory factors and stimulated that of anti-inflammatory ones in cultured adipocytes [125].

An extract facilitated wound healing properties in a clinical trial [126], and was effective for the treatment of psoriasis, lowering TNFα levels in dermis, endothelial, and dendrite cells [127]. An ethanolic extract prevented lipid peroxidation in neutrophils of patients with Behcet's syndrome [128]. An oil extract was effective for the prevention and treatment of pressure sores [129]. Wound-healing properties of *H. perforatum* extracts were confirmed in different models [130–133] including diabetic animals [134–136]. An oil extract prevented the narrowing of the oesophageal lumen caused by burn injuries [137], and had anti-inflammatory, anti-angiogenic, and anti-fibroblastic effects when applied after corneal alkali burns [138]. Hyperforin reduced the migration of fibroblasts in 2D and 3D models of artificial skin and was proposed for the treatment of hypertrophic scars [139].

Hypolipidaemic and hypoglycaemic properties

Hypolipidaemic and hypoglycaemic properties have been discovered for extracts of the aerial parts of *H. perforatum* [141–143]. A hydroalcoholic (50%) extract of the whole plant at the doses of 100 and 200 mg/kg of body weight per day for 15 days exhibited hypocholesterolaemic properties [144], and similar effects were observed for an aqueous extract of the aerial parts at the dose of 300 mg/kg for 60 days [145]. A methanolic extract and hyperforin prevented pancreatic β-cells from damage by the cytokines iNOS, CXCL9, CXCL10, and COX2, which is associated with the development of type I diabetes [146]. It was found that excessive intake of *H. perforatum* flower extract, hypericin, and hyperforin could aggravate diabetes and obesity by inhibiting the differentiation of preadipocytes and inducing insulin resistance in mature fat cells [147].

Effects of *H. perforatum* in genitourinary system disorders

A powder of shoots at the dose of 200 mg/kg (8 weeks) in diabetic nephropathy showed a nephroprotective effect [147]. A methanolic extract of the aerial parts of *H. perforatum*, hypericin, and hyperforin exhibited spasmolytic activity and modulated detrusor contractile activity in isolated urinary bladder; for hypericin, this effect was associated with an increase in plasma membrane depolarization, and for hyperforin, with a stimulatory effect on the cholinergic system [149]. A hydroalcoholic extract of leaves reduced the size and number of ethylene glycol-induced renal calculi [150].

Clinical trials found out that a powder of St. John's wort given at the doses of 270–330 μg for 2 months reduced hot flashes, menopausal symptoms, and depression [151], and the extract was effective for the treatment of premenstrual syndrome [152]. In an *in vitro* experiment, the extract (25 μg/mL) and hypericin (7.5 and 75 ng/mL) increased calcium concentration in JEG-3 placental cells [153]. The extract intake (100 mg/kg and 300 mg/kg) from mating till delivery prolonged foetal development and damaged foetal liver due to oxidative stress [154]; at the same doses, the extract worsened ovarian function and decreased fertility [155].

Effects of *H. perforatum* in maxillofacial injuries

An aqueous extract of the aerial parts activated a bone tissue regeneration in the orthopaedically expanded premaxillary suture, which is performed in orthognathic surgery [156]. Another experiment proved a standardized methanolic extract to restore mandible bone tissue in a stress model [157]; an ethanolic extract activated dental pulp regeneration [158]), and an oil extract improved bone healing after xenograft–implantation [159].

[1] It was reported that the cytotoxicity of photodynamic hypericin was higher for amelanotic A375 melanoma cells in comparison with pigmented Mel-1 cells; in this regard, melanin was suggested to play a role in the chemoresistance of melanoma cells (Sharma, Davids, 2012b).

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Other effects

Several *H. perforatum* extracts prevented acetaminophen-induced liver injury [160, 161], a petroleum ether leaf extract had protective effects in a hepatic ischaemia-reperfusion model [162], and another extract accelerated hepatic clearance of technetium-99 [163]. Certain fractions of a water/ethanol extract of the aerial parts of *H. perforatum* had spasmolytic, bronchodilator, vasorelaxant and cardiotropic activities [164]; *H. perforatum* polysaccharides and a methanolic seed extract showed antioxidant properties [165, 166]. It is assumed that the antioxidant properties underly the photoprotective and anti-inflammatory effects of hyperforin on skin tissues [167]. At the doses of 250 and 500 mg/kg, a *H. perforatum* dry extract reduced binge eating episode frequency [168]. A leaf extract had antimutagenic properties [169]; the effects of hyperforin were described as antigenotoxic [170] and DNA-protective [171] in different *in vitro* models. An ethanolic extract of *H. perforatum* stimulated human osteoblast-like MG-63 cell proliferation in osteoporosis induced by ovariectomy [172]. A hydroalcoholic extract (110 mg/kg for 2 weeks) enhanced cellular immunity [173], and a methanolic extract of the aerial parts, dissolved in olive oil, prevented the development of myringosclerosis after myringotomy [174]. The β-diketone 2,6,9-trimethyl-8-decene-3,5-dione, hyperforatins B, D, and F, 15-epi-hyperforatin D, and 32-epi-hyperforatin E inhibited an acetylcholinesterase activity [175, 176], and a methanolic extract stimulated hepatic and renal activities of the cytochromes CYP3a and CYP2c [177].

CONCLUSION

Despite the popularity of *H. perforatum* as a plant with an antidepressant-like activity, intensive research work continues to be carried out to elucidate the molecular mechanisms of the actions of extracts and individual compounds in disorders of the nervous system. Studying its antibacterial, antiviral, and cytotoxic activity may also open up some great prospects, along with determining the possibility of using St. John's wort in metabolic disorders, genitourinary disorders, and other fields of medicine.

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AUTHORS' CONTRIBUTION

A.L. Budantsev – writing Introduction and Antibacterial, antiviral, antiprotozoal activity, Antitumor and cytotoxic properties, Analgesic, anti-inflammatory and wound-healing properties, compiling the list of references; I.V. Varganova – compiling of Antibacterial, antiviral, antiprotozoal activity, Antitumor and cytotoxic properties, Analgesic, anti-inflammatory and wound healing properties, Hypolipidemic and hypoglycemic properties, other effects, translation of the text into English, compiling the list of references; V.A. Prikhodko – compiling of Antidepressant activity, Neuroprotective activity, Nootropic activity, Antiepileptic activity, Anxiolytic activity, Effects of *H. perforatum* in genitourinary system disorders, Effects of *H. perforatum* in maxillofacial injuries, translation of the text into English, compiling the list of references; S.V. Okovity – compiling of Antidepressant activity, Neuroprotective activity, Nootropic activity, Antiepileptic activity, Anxiolytic activity, Effects of *H. perforatum* in maxillofacial injuries, Effects of *H. perforatum* in maxillofacial injuries.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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REVIEWS

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PHARMACEUTICAL SERVICES: STATUS AND DEVELOPMENT TRENDS

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The aim of the review is to provide an analysis and generalization of the main directions of research in the sphere of pharmaceutical services, and their characteristics associated with the determination of their main development trends.

Materials and methods. For the analysis, the information store on the basis of scientific publications by Russian and foreign scientists, devoted to research in the field of pharmaceutical services (PSs), has been used. The search for publications was carried out in the open and accessible sources of the latest twenty years (the retrospective period of 2001–2021), located in scientific and technical libraries of institutions, as well as in electronic databases: Elibrary, Medline / PubMed, Cochrane Library, Scopus, CyberLeninka, Google-Academy, J-stage. When forming the information array, the search for publications was carried out according to the following requests: pharmaceutical services (pharmaceutical care services), the provision of pharmaceutical services, the quality of pharmaceutical services. For the conceptualization of the study, 87 scientific publications obtained as a result of information retrieval, have been used.

Results. In the course of the study, a logical and structural analysis of the main directions in which research in the field of providing PSs in our country is developing, has been carried out. The main trends in the study of the providing services' activity in the sphere of drug circulation, are characterized. A comprehensive analysis of the category of "pharmaceutical service" has been carried out. The terminological content of this concept, the groups of features characterizing the economic and social essence of educational institutions have been generalized, and the most characteristic features that make up the structure and content of educational institutions, have been identified. The existing approaches to the development of the nomenclature and types of PSs have been analyzed and the systematization of pharmaceutical works and services using the process approach, have been proposed by the authors.

Conclusion. The conducted study indicates the presence of several directions in the development of research in the field of providing PSs, aimed at improving the quality of services for the population in pharmaceutical organizations. However, the most important role in the research is assigned to the study and assessment of the quality of educational institutions, the development of approaches to its optimization. As evidenced by the results of the analysis and generalization, the most successful activity in the provision of services in the field of drug circulation requires the implementation of a process approach and the implementation of Quality Management Systems (QMSs).

Key words: literature review; pharmaceutical aid; pharmaceutical service; quality of pharmaceutical services; pharmacy organization; pharmaceutical retailer

Abbreviations: MPs – medicinal preparations; MRs – Medicine remedies; QMS – quality management system; SOP – Standard Operation Procedure; PAs – Pharmaceutical assortment; PA – pharmaceutic aid; PSs – pharmaceutical services

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ФАРМАЦЕВТИЧЕСКИЕ УСЛУГИ: СОСТОЯНИЕ И ТЕНДЕНЦИИ РАЗВИТИЯ

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Цель. В обзоре приводится анализ и обобщение основных направлений исследований по изучению сферы фармацевтических услуг и их характеристике, определению основных тенденций развития.

Материалы и методы. Для проведения анализа авторами использован информационный массив, сформированный на базе научных публикаций российских и зарубежных ученых, посвященных исследованиям в области предоставления фармацевтических услуг (ФУ). Поиск публикаций осуществлялся среди открытых и доступных источников последних двадцати лет (период ретроспекции 2001–2021 гг.), размещенных в научно-технических библиотеках учреждений, а также в электронных базах данных: Elibrary, Medline/PubMed, Cochrane Library, Scopus, Киберленинка, Google-академия, J-stage. При формировании информационного массива поиск публикаций осуществлялся по запросам: фармацевтические услуги (pharmaceutical services, pharmaceutical care services), предоставление фармацевтических услуг, качество фармацевтических услуг. Для построения концептуальной основы исследования мы использовали 87 научных публикаций, полученных в результате информационного поиска.

Результаты. В ходе исследования проведен логический и структурный анализ основных направлений, по которым развиваются исследования в области предоставления ФУ в нашей стране. Охарактеризованы основные тенденции изучения деятельности по предоставлению услуг в сфере оборота лекарственных средств. Проведен комплексный анализ категории «фармацевтическая услуга», в том числе раскрыто терминологическое содержание данного понятия, обобщены группы признаков, характеризующих экономическую и социальную сущность ФУ, выявлены наиболее характерные особенности, составляющие структуру и содержание ФУ. Авторами проведен анализ существующих подходов к разработке номенклатуры и видов ФУ, предложена систематизация фармацевтических работ и услуг с использованием процессного подхода.

Заключение. Проведенное исследование свидетельствует о наличии нескольких направлений развития исследований в области предоставления ФУ, направленных на повышение качества обслуживания населения в фармацевтических организациях. Однако, наиболее важная роль в исследованиях отводится вопросам изучения и оценки качества ФУ, разработке подходов к его оптимизации. Как свидетельствуют результаты проведенного анализа и обобщения, наиболее успешная деятельность по предоставлению услуг в сфере оборота лекарственных средств требует реализации процессного подхода и внедрения систем менеджмента качества (СМК).

Ключевые слова: обзор литературы; фармацевтическая помощь; фармацевтическая услуга; качество фармацевтических услуг; аптечная организация; субъект розничной торговли фармацевтическими товарами

Список сокращений: ЛП – лекарственные препараты; ЛС – лекарственные средства; СМК – система менеджмента качества; СОП – стандартная операционная процедура; ТАА – товары аптечного ассортимента; ФП – фармацевтическая помощь; ФУ – фармацевтические услуги

INTRODUCTION

The process of providing the population with PSs is closely connected with the field of PSs, which, along with pharmaceutical products and information about them, are integral components of PSs. PSs are important in the health care system. Currently, in international practice, the term “pharmaceutical services” refers to all types of the services provided by pharmaceutical specialists to solve the problems of pharmacotherapy in pharmacy organizations [1]. The provision of such services is the result of the activities of a pharmaceutical organization and implies the implementation of a number of sequential labor functions, actions and operations of pharmaceutical personnel aimed at organizing and providing high-quality, timely and affordable PSs to the population.

A modern market of pharmaceutical services is a system of economic relations that have developed be-

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tween the consumer and the provider of the demanded services. The area of a financial management is very extensive and includes both manufacturers of pharmaceutical products, wholesale and retail trade entities, and organizations of various levels that exercise supervision and control in the field of medicinal preparations and pharmaceutical assortment (PAs) circulation [2]. The effectiveness of providing ultimate customers with PSs is determined by the organization level of the entire system of production and sales of PSs, while the influence of each economic entity involved in this area, is also significant. Taking into account the existing duality of the socio-economic importance of PSs, a proper management (a rational organization of the PSs system) largely determines the quality of providing the population with PSs. The appropriate level of the customer service directly contributes to strengthening the competitiveness and ensuring the financial and economic stability of pharmaceutical organizations.

It should be noted that in the provision of PSs, the most important role is assigned to the pharmaceutical retailers (hereinafter the retailer), since they are the central link in the logistics chain for the promotion of pharmaceutical goods to the ultimate consumer and complete the process of distribution. In addition, they are a connecting element between the attending physician and the patient. In this regard, not only the type and quality of the provided PSs and, as a consequence, satisfaction of the requirements and expectations of consumers, but also the effectiveness of pharmacotherapy in general, depend on work organization of retail trade entities [3].

Taking this circumstance into account, and to determine approaches to the research of PSs in pharmacy retail organizations, a detailed analysis of scientific publications has been carried out.

THE AIM of the review is to provide an analysis and generalization of the main directions of research in the sphere of pharmaceutical services, and their characteristics associated with the determination of their main development trends.

MATERIALS AND METHODS

For the analysis, the information store on the basis of scientific publications by Russian and foreign scientists, devoted to research in the field of pharmaceutical services (PSs), has been used. The search for publications was carried out in the open and accessible sources of the latest twenty years (the retrospective period of 2001–2021), located in scientific and technical libraries of institutions, as well as in electronic databases: Elibrary, Medline/PubMed, Cochrane Library, Scopus, Cyberleninka, Google-academy, J-stage. When forming the information array, the search for publications was carried out according to the following requests: pharmaceutical services (pharmaceutical care services), the provision of pharmaceutical services, the quality of pharmaceutical services. The results of the information search contained 237 literature sources, including scientific articles, monographs, study guides, publications from the abstracts of candidate and doctoral theses defended by Russian scientists during the specified period. The list of the sources was manually checked by the researchers, and the selection was based on the titles of the publications. For the conceptualization of the study, 87 scientific articles by authors from Russia and other countries, the titles of which contained the term “pharmaceutical services”, have been used.

RESULTS AND DISCUSSION

In the course of conducting a content analysis of Russian publications selected according to the results of search queries, it was found that, depending on the field of study chosen by the author, the array of publications devoted to the study of PSs can be divided into several groups:

1. Studying the socio-ethical aspects in providing PSs. This group of publications covers the issues of relationships between suppliers and consumers of PSs, increasing consumers’ loyalty to the pharmacy network, studying the social role of pharmaceutical specialists in providing PSs and implementing the concept of “pharmaceutical service”. The works by S.N. Fomicheva are devoted to the research in this area. [4], Yu.O. Agadzhanyan et al. [5], A.D. Sibireva [6, 7], I.M. Razdorskaya, Zanina I.A. [8].
2. Studying the matters of quality management in the field of providing PSs – this group of publications includes the results of studies on assessing the effectiveness and ways of optimizing the quality of certain PSs types provided in pharmaceutical organizations – A.M. Gosudarev [9], K.A. Livshits [10], P.A. Lisovskiy [11], F.N. Bidarova [12], R.G. Dyachenko et al. [13], D.A. Blokhina [14, 15].
3. Studying individual areas of pharmaceutical services – a group of publications includes the results of marketing research in the provision of information, consulting and additional services provided by pharmaceutical organizations – E.A. Fedina [2], N.O. Karabintseva [17], L.N. Tsarakhova [18], A.Kh. Gaisarov [19, 20], S.V. Semenova [21, 22].
4. 4. Studying the local market of pharmaceutical services and goods – a group of publications brings together the results of studies of regional aspects of providing PSs when selling (dispensing) certain types of goods, for example, cosmetics, pharmaceutical products for dentistry, over-the-counter products, herbal medicines, etc.: S.V. Kononova [23]; Yu.N. Bogdanova [24]; N.A. Samarova. [25], G.M. Fedotov [26], I.V. Popov et al. [27].
5. Studying the economic aspects of PSs – the subject of research in this segment was the organizational and economic relations arising in the process of functioning the pharmaceutical market subjects,

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the problems of ensuring the PSs economic security, the problems of promoting PSs and the formation of a competitive environment –T.V. Zernova [28], S.V. Esaulov [29], A.I. Basargina [30].

Taking into account the data of the conducted content analysis of the literary sources, it has been established that in the Russian pharmaceutical science the problems of PSs are thoroughly investigated and actively developed. At the next stage of the study, it was necessary to find out the structure of such studies and the main trends in their development.

The main research trends of Russian scientists in the field of providing PSs

The results generalization of the content analysis of the information array and the study of scientific publications devoted to the problems of providing PSs, made it possible to identify a number of characteristic trends in the authors' studies. Most of the directions in which Russian research in the field of financial education is developing, coincide and can be combined into the following 4 groups:

1) Clarification of the terminological content of the "pharmaceutical services" concept;

2) Studying the essence and distinctive characteristics inherent in PSs;

3) Development of the nomenclature and types of PSs;

4) The development of methodological approaches to optimizing the quality of PSs, aimed at increasing the degree of consumer satisfaction, including the following: assessing the quality and efficiency of providing PSs, developing standards for the provision of PSs certain types, developing approaches to optimizing the work of pharmaceutical specialists in the provision of PSs, etc.

A schematic list of the areas reflecting the current research trends in PSs in our country is shown in Fig. 1.

Based on the data of the literary sources content analysis and taking into account the identified areas of research, an integrated approach was applied to the study and assessment of the category of "pharmaceutical service". It makes it possible to most fully reveal the terminological content of this concept, to substantiate the groups of features that characterize their economic and social essence, to conduct a systematization of pharmaceutical works and services and to determine the approaches to assessing and optimizing the PSs quality.

Comprehensive analysis of the "pharmaceutical service" category

1. Clarification of terminological content of the "pharmaceutical service" concept

To conduct a comprehensive analysis of this category, it was first of all necessary to clarify the terminological content of the "pharmaceutical services" definition. While studying foreign literature, it was found out that the term "pharmaceutical services" was originally used by Helper C.D. and Strand L.M. to identify all types of services a pharmacist needs to solve patients' pharmacotherapy problems, from providing them with drug information and to drug distribution. According to the authors, timely and high-quality provision of such services is necessary to guarantee the PSs provision [31–33].

According to the definition given by WHO specialists, pharmaceutical services refer to all types of services provided by pharmaceutical personnel to support and provide patient-centered PSs. Herewith, different areas of pharmaceutical specialists' activities are taken into account (administrative and regulatory bodies, professional associations, public health, educational institutions). Thus, in addition to providing pharmaceutical products, PSs include information, education and communication to promote public health, drug information and counseling, regulatory services, education and training of personnel.[1]

Similar concepts of PSs with minor differences are accepted in different countries of the world. For example, in the United States, PSs must be consumer-oriented, including patients, healthcare professionals and third parties. Despite the diversity of PSs, they all share the same philosophy and goals, namely, "the responsible provision of drug therapy in order to achieve specific results that improve the quality of life of patients [34–35].

In the Brazilian health care system, the implementation of PSs is generally focused on the activities intended for patients. At the same time, PSs are a set of systematically performed actions aimed at strengthening, protecting and restoring the health of the population by ensuring the availability and rational use of drugs [36–39].

The concept of the pharmaceutical services sector in Slovakia defines PSs as the basic part of pharmacy, the main task of which is to provide pharmaceutical aid. PSs is a set of professional actions of a pharmacist aimed at ensuring the safety of drugs, as well as optimizing effective, safe and high-quality pharmacotherapy [40].

In Australia, a professional pharmaceutical service is an action or a set of actions taken in or organized by a pharmacy, carried out by a pharmacist or another healthcare professional who applies his or her specialized knowledge personally or through an intermediary to a patient (a client, community) in order to optimize the pharmaceutical aid delivery process to improve health outcomes [41–42].

In our country, traditionally, the term "pharmaceutical services" refers to all types of services provided by pharmaceutical specialists to solve the problems of patient pharmacotherapy [19]. However, in the works of domestic scientists, in the course of a comparative analysis of scientific publications, various approaches used to concretize and clarify this term, have been found out. The results of the analysis are presented in Table 1.

[1] Counseling, concordance, and communication: innovative education for pharmacists. The Hague: International Pharmaceutical Federation (FIP) and International Students' Federation; 2005. Available from: // www.fip.org/. (Date of access 03 Sep 2019).

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Thus, the analysis of the definitions structure of "pharmaceutical services" indicates that very often this term is understood as a certain form of activity in drug provision, drug care, drug service.

Thus, variations in the definition of the term "pharmaceutical services" proposed by A.V. Soloninina. and L.V. Moshkova, contain such a connotation as "a set of measures" related to providing consumers with necessary pharmaceutical products. In their studies, N.B. Dremova, A.I. Ovod, E.A. Korzhavykh, L.N. Geller consider PSs as "a certain type of professional activity", and A.S. Nemchenko. and A.L. Panfilova when defining this term, are guided by the result of such activities. P.A. Lisovskiy and K.A. Livshits use the term "process", and according to S.A. Smirnova, S.V. Kononova and G.A. Oleinik, PSs mean "work". Considering the economic essence of PSs, A.M. Gosudarev and C.V. Esaulov attributed them directly to the "types of services provided by pharmaceutical organizations". N.O. Karabintseva et al, characterize PSs delivery activities as "the result of interaction between PSs consumers and pharmaceutical specialists".

Comparative characteristics of the features used by different authors in the terminological substantiation of the "PSs" concept, are presented in Table 2.

Thus, the provision of PSs is primarily focused on meeting the needs of the consumers (patients) and is aimed at providing his drug treatment, preservation and maintenance of health. The content analysis showed that such a meaning is embedded in all the definitions of PSs proposed by leading Russian scientists. In addition, common attributing features are the process of interaction between the consumer and the pharmaceutical specialist and the provision of the necessary information during the course of pharmaceutical consulting.

In the term "pharmaceutical services" the authors often highlight such a common feature as "meeting the needs of the population" [3, 11, 28, 43, 45]. Most of the definitions of PSs include and use such necessary components as "provision of pharmaceutical care", "drug treatment of the patient" [3, 28, 29, 31, 43, 46, 47]. However, some authors, when interpreting the term "PSs", clarify the presence of a commercial component in the provision of pharmaceutic aid [28, 46].

The judgments of a number of domestic researchers regarding the impact of PSs on the quality of a pharmaceutical product through information and consulting support and ensuring compliance with the rules and regulations for its handling, are also of interest [23, 44]. In the authors' opinion, this definition emphasizes the result of a service as a change in the quality of the object under consideration. This definition is more in line with modern ideas that have developed in the economic theory about the essence of the services. From this point of view, PSs should include various labor operations of the personnel of a pharmaceutical organization that contribute to the formation and preservation of the quality of the products sold. [23, 44].

In Geller L.N.'s definition, the effect of "useful action of PSs" is reflected, and PSs are considered as a set of some attributes (the benefits that the consumer is looking for), and it is the attributes that "create services" and the degree of satisfaction with them [45]. The useful effect of the activity, forms the consumer value i.e. usefulness of the service. Consequently, the beneficial effect of PSs is the result of living or materialized labor. Thus, pharmaceutic services exist as labor (activity, work) embodied in a specific material object (product) in the form of a useful effect of living labor consumed directly in the service process.

The results obtained make it possible for us to conclude that the term "pharmaceutical service" in the works of domestic researchers, was considered from different points of view and until now there is no unambiguous terminological definition of PSs. Most often, PSs are viewed as an activity (process, set of activities) aimed at providing pharmaceutic aid and providing consumers with the necessary pharmaceutical products. A comparative analysis of publications showed that there is some similarity in the definitions proposed by various authors. But this circumstance does not indicate the consistency to the definition of the term "pharmaceutical service". In turn, the lack of a common language in relation to PSs, to a certain extent, complicates further research in this area.

The results of studying the available approaches to the definition and the essence of the concept of "PSs" indicate that the unambiguous identification of the term is difficult due to the significant divergence of opinions of Russian scientists regarding the very essence of the PSs. This circumstance suggests that the existing ambiguity is a consequence of terminological uncertainty in the theory of services.

In this regard, it is necessary to first dwell on the consideration of the concept of "service". Despite the fact that service provision activities play an important role in the service sector, there is no single and generally accepted definition of the term "service" in the economic theory. Due to its heterogeneity, the term "service" is interpreted by scientists in different ways and carries many semantic shades, including everyday and scientific ideas [48]. During the content analysis of the scientific publications on the topic under consideration, it was found out that the term "service", in most cases, is used in the context of concepts that reflect the results of the production process: "economic product" ("economic benefit" "produce", "goods"), "work". As a rule, a service is understood as the performance of a certain activity or a set of activities aimed at meeting the needs of others.

In the specialized literature and normative legal documents, such concepts as "occupation" and "service" are often used together, but the current legislation does not

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have a clear definition of these terms. These concepts are so inseparable and close to each other that until now there is no consensus on their delimitation, and sometimes these concepts are identified by different authors.

The differentiation of the concepts of "pharmaceutical occupations" and "PSs" is given in the papers by V.N. Mikhailova et al. [47]. In their opinion, PSs are a set of measures aimed at providing pharmaceutical assortment (PAs) consumers with the necessary information. The authors comprise all types of work aimed at the implementation of PSs and pharmaceutical activities on the whole [50].

In our opinion, the difficulty of differentiating these concepts is also confirmed by the fact that the work performed is a flow of work, aimed at obtaining the final result in the form of useful activities and subject to assessment and payment [49]. However, no service is possible without performing the corresponding production activity (work), and the result of the service cannot be separated from the business processes performed during its provision. Thus, the concepts of "service" and "work" are closely interrelated, however, a service is a more complex phenomenon, it has a purpose, contains a certain set of labor actions and is accompanied by a certain useful effect, due to which the service becomes demanded by the consumer.

According to the interpretation of GOST R 50646-2012 "Services to the population. Terms and definitions", a service can be represented as both the service provider's own activity to meet the needs of the consumer, and the direct interaction between the contractor and the consumer during the goods release. A similar interpretation of the term is presented in the ISO standards, according to which a service is the result of the supplier's internal activities aimed at meeting the needs of the consumer and direct interaction between the consumer and the supplier.

Thus, it can be concluded that the concept of "service" includes some internal activity of the performer (work), the process of interaction between the contractor (work) and the consumer (service), and the result of the service demanded by the consumer (a useful effect of the service). In turn, the result of the service manifests itself in the form of a useful effect, in the first case – in the form of a transformed work, and in the second – in the form of serving the consumer. It is this effect that has a use value. In addition, it should be noted that the beneficial effect of most services is consumed in the process of serving the consumer, simultaneously with their provision [50–52].

Among a lot of approaches to define a service, the most acceptable, in the authors' opinion, is the approach of the American scientist T. Hill, according to which the result of a service activity is a change in the quality of the object that the service itself is aimed at [55]. According to this approach, the activity of providing services can be considered as a process of changing the state of the subject by one of the participants in economic relations, if they have a voluntary agreement.

Thus, it has been established that the provision of PSs is aimed at formation of use value and maintaining the consumer characteristics of pharmaceutical products in the process of their promotion from manufacturer to consumer. The process of providing PSs itself implies both information and consulting support of pharmaceutical products during their transfer to the consumer, as well as changing and (or) preserving the condition of pharmaceutical products, ensuring their good quality in the process of circulation.

In the process of PSs implementation, on the one hand, a pharmaceutical product is sold, and on the other hand, a service is provided as a consequence of the transformed work. The specificity of the pharmaceutical industry is the fact that all goods, services and work in it are interrelated and inseparable from each other. The algorithm of the PSs rendering process is shown in Fig. 2.

Taking into account various terminological interpretations of the "service" concept in the definition dictionary of the Russian language, GOST R 50646-2012, the reference book of ISO and WHO terms, publications by scientists and the results based on the author's own research, the following working definition of the term has been proposed: a pharmaceutical service is a set of professional and continuously implemented labor operations to promote pharmaceutical products to the ultimate consumer to meet his needs of being physically, socially and spiritually healthy.

2. Study of the essence and distinctive characteristics of PSs

In their works, the authors give PSs the following main characteristics:

- impalpability, continuity of production and consumption, variability of quality, inability to store [44];
- a high use value (it contributes to the maintenance and preservation of human health); delayed and differentiated nature of the result; a special form of payment (financing is carried out both by the state and by the consumer); pronounced territorial boundaries of the PSs market; the state regulation of the PSs market [23];
- a high social significance; a high degree of individualization of PSs in accordance with the requirements of the consumer; the importance of the role of a specialist producing PSs, which is due to the high scientific content of the pharmaceutical sector and the lack of consumer awareness [11];
- elusiveness, immateriality, simultaneous manufacturing and consumption, the dependence of the quality of the service on the level of qualification of the contractor and the complexity of the measurement [2].

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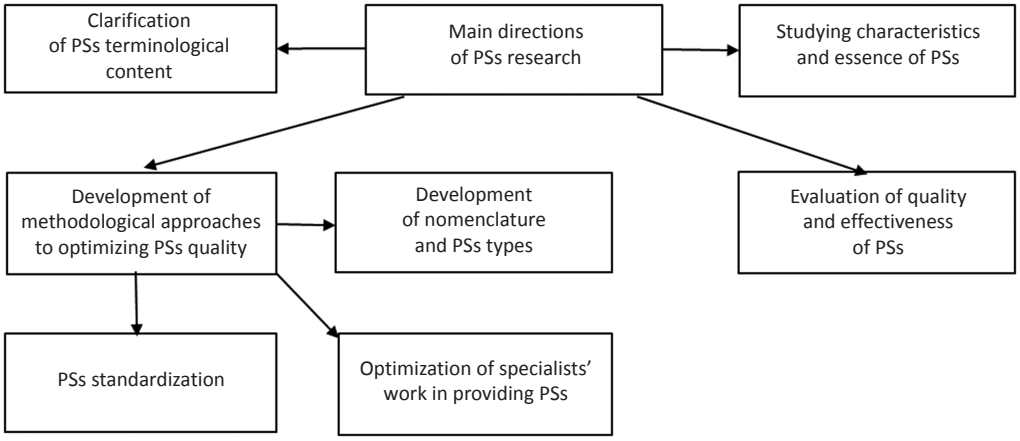

Figure 1 – The main directions of modern research in pharmaceutical services

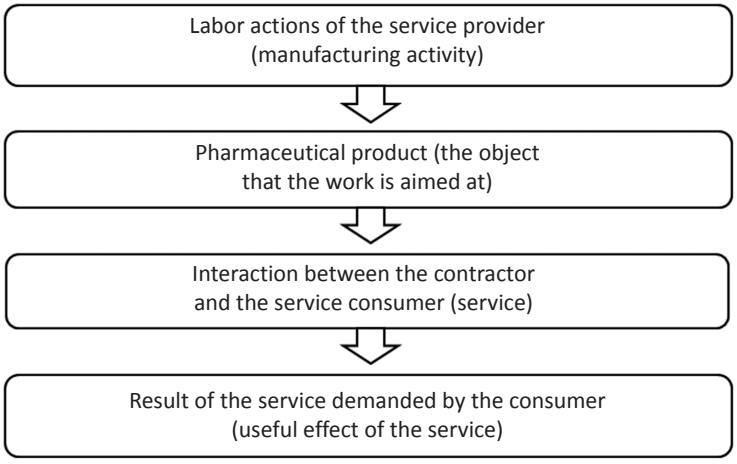

Figure 2 – Algorithm of the process of providing pharmaceutical services in a retail entity

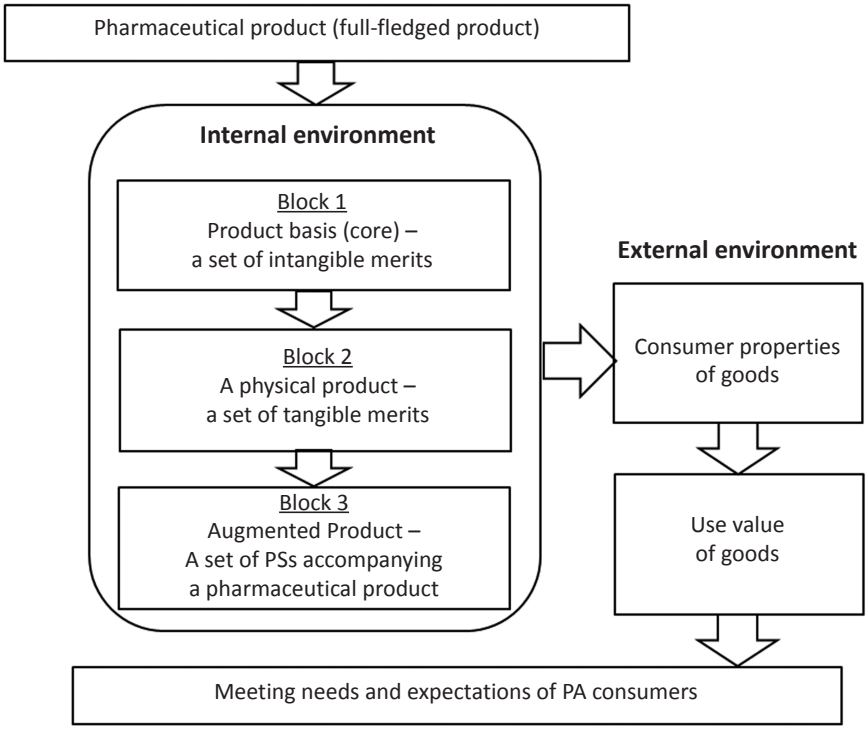

Figure 3 – Marketing conceptual model of a pharmaceutical product

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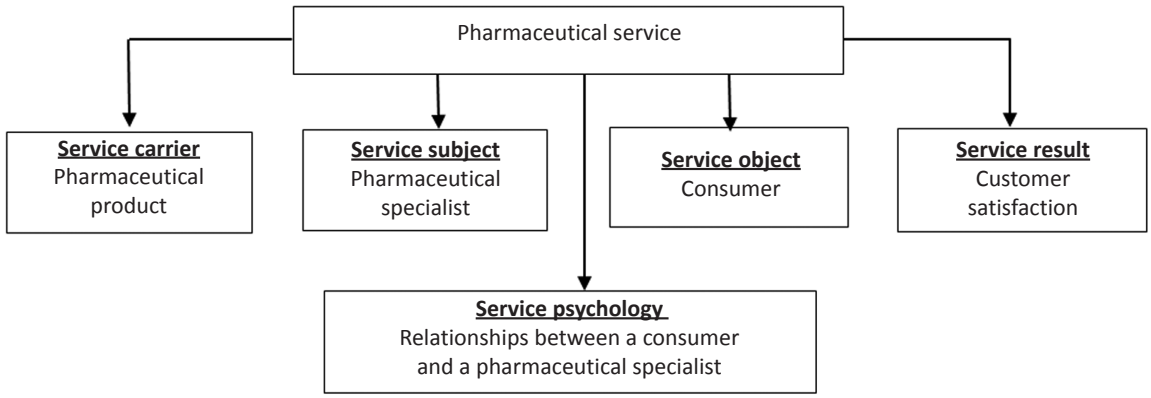

Figure 4 – The structure and content of a pharmaceutical service

Table 1 – Analysis of publications on the terminological definition of the "pharmaceutical service" term

Author/Source	Research period	Interpretation of the term "pharmaceutical services"
AV Soloninina [43]	2001	A set of measures for the provision of medicinal, therapeutic and prophylactic, diagnostic agents, medical devices, parapharmaceutical products in the health care system.
SV Kononova, GA Oleinik [23]	2003	The work, in the process of which a new drug is not created, but its quality may change due to the information and consulting support and ensuring compliance with the rules and regulations for its circulation. The process of selling services is accompanied by the sale of goods and the provision of services, i.e. goods and services are interconnected.
AM Gosudarev [9]	2003	The services provided to the population and healthcare organizations by legal entities – wholesalers, pharmacies of any form of ownership and individuals engaged in entrepreneurial activities.
KA Livshits [10]	2004	The process of promoting a pharmaceutical product from a manufacturer to an intermediate or final consumer.
SA Smirnova [44]	2005	The aggregate quality product of research, production, information, advisory, medicinal and other purposes necessary for the consumer and the types of work during which a new medicinal product is not created, but its quality may change due to the information and consulting support and ensuring compliance with the rules and regulations for its circulation.
LN Geller [45]	2008	The activity of a pharmacy organization on the formation of a set of pharmaceutical services attributes of a functional, aesthetic, emotional and other kinds of nature, the results of which are expressed in a beneficial effect that satisfies a person's needs to be physically, socially and spiritually healthy.
TV Zernova [28]	2008	A set of actions on meeting the needs for maintaining a given level of consumer life quality, based on establishing a balance of commercial and social significance through drugs and information and pharmaceutical services.
PA Lisovsky [11]	2009	A process aimed at meeting the needs of the population in pharmaceutical products by obtaining information about them and then purchasing them in pharmacies, as well as within the framework of medical and preventive care.
NB Dremova, AI Ovod, EA Korzhavykh [3]	2009	A form of a pharmaceutical activity in which a specific need of a patient or medical institution is satisfied.
AS Nemchenko, AL Panfilova [46]	2010	Pharmaceutical assistance provided to the population at the service (commercial) level as a result of the professional activities of pharmaceutical specialists in order to preserve and maintain the citizens' health.
NO Karabintseva, LV Moshkova, MP Boyko [17]	2010	The result of the interaction of pharmaceutical specialists and direct consumers of these services, who can be both patients and doctors.
SV Esaulov [29]	2012	The services provided by pharmacy organizations in the production process, dispensing, consultations on the use of pharmaceutical products.
VN Mikhailova et al [47]	2012	A set of measures aimed at providing drug consumers with pharmaceutical goods and necessary information.

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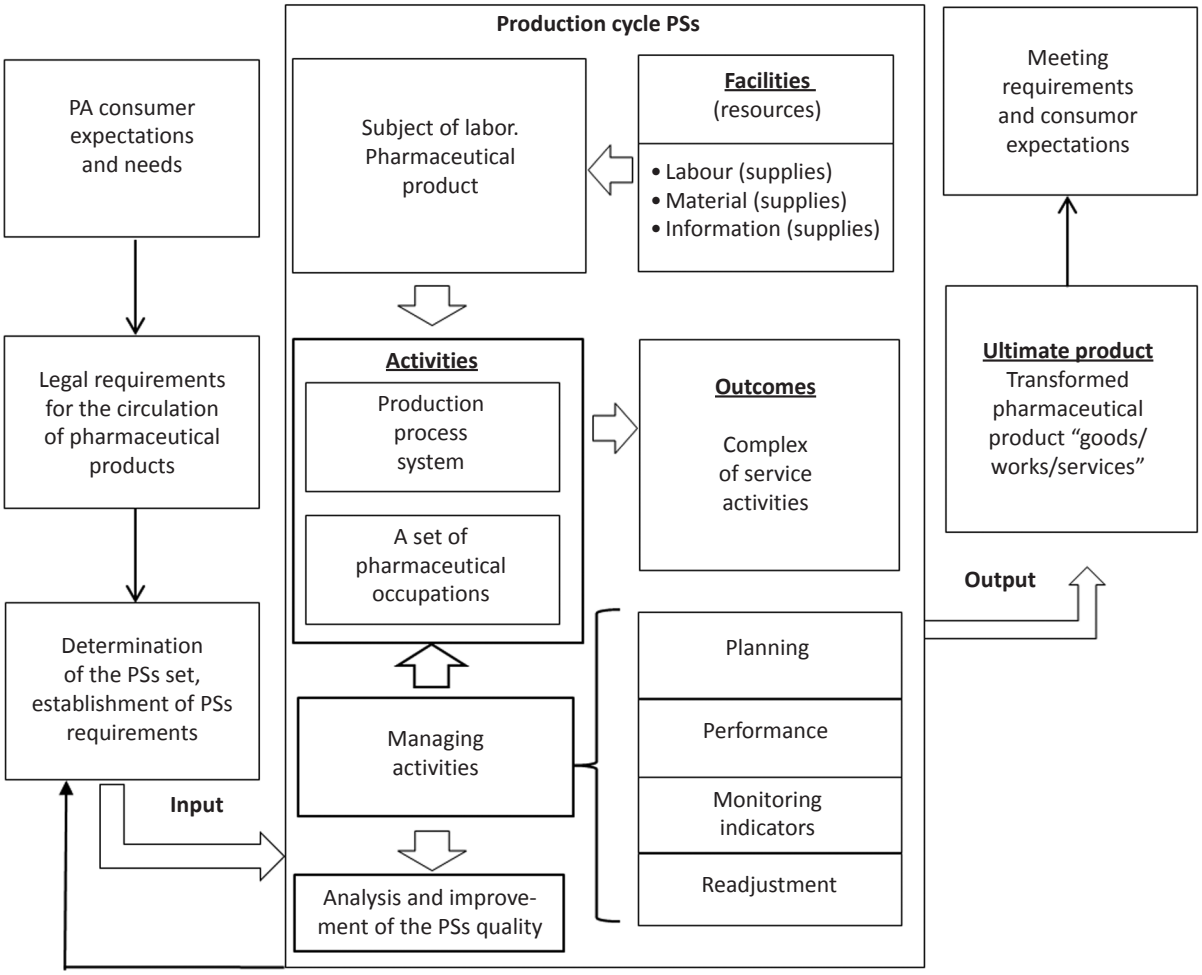

Figure 5 – Model of the process for providing pharmaceutical services in the retail subject

Table 2 – Comparative characteristics of the features used in the terminological substantiation of the term "pharmaceutical services"

Definition signs	Source														
	3	9	10	11	17	23	28	29	31	43	44	45	46	47	WHO
A set of activities							+		+	+				+	
Activities	+											+	+		
Process			+	+											
Work						+					+				
Services		+						+							
Product											+				
Result of the interaction between the consumer and a pharmaceutical specialist		+		+	+	+	+	+							
Result of professional activities		+			+						+		+	+	
Provision with pharmaceutic aid, drug treatment				+			+	+	+	+			+	+	+
Meeting the needs	+			+			+				+	+			
Impact on product quality						+					+				
Product promotion (selling, distribution)			+						+						
Providing information, consulting				+		+	+	+	+		+			+	
Commercial level of pharmaceutic aid		+					+						+		

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Table 3 – Groups of features characterizing PSs

Item number	Feature group	Service characteristics
1	Attitude towards the product	Monetary-dependent services
2	Attitude towards the consumer	Personal services Public services
3	Attitude towards the society	Socially significant services
4	Attitude towards perception	Intangible consumer services
5	Attitude towards the contractor	Professional services inseparable from the contractor
6	Attitude towards quality	Conditionally constant quality services
7	Attitude towards the degree of consumer engagement	Internal activity services (non-contact type of service) Services of interaction with the consumer (contact type of services)
8	Attitude towards duration of exposure	Fragile services
9	Attitude towards the production processes and a service consumption	Services that are inseparable from production activities, retained in the "goods/works/services" complex and implemented at the time of the product sale

Table 4 – The Pharmacy Practice Activity Classification (PhPAC)

Code	Classification of pharmacy practice activities
A	**Ensuring appropriate treatment and outcomes**
A1	Ensuring appropriate pharmacotherapy
A2	Ensuring patient's understanding/adherence to his or her treatment plan
A3	Monitoring and reporting oucomes
B	**Dispensing medications and devices**
B1	Processing the prescription or medical order
B2	Preparing the pharmaceutical product
B3	Deliverying the medication or device
C	**Health promotion and disease prevention**
C 1	Delivering clinical preventive services
C 2	Surveillance and reporting of public health issues
C 3	Promoting safe medication use in society
D	**Health system management**
D 1	Managing the practice
D 2	Managing medications through the health system
D 3	Managing the use of mediations within the health system
D 4	Participation in research activities
D 5	Engaging in interdisciplinarycollaboration

Table 5 – Systematization of pharmaceutical occupations and services using a process approach

Item number	Category of occupations and services	Production processes accompanying the provision of PSs	Types of work and services performed
1	Occupations and services of strategic importance	Development processes are a set of production processes that ensure the improvement of the quality of services provided	1. Occupations and services for planning activities; 2. Occupations and services for monitoring and analyzing activities; 3. Occupations and services for improving activities.
2	Organizational and managerial occupations and services	Managing processes are a set of production processes aimed at organizing the work of a pharmacy organization, ensure a proper production of all types of services.	1. Occupations and services for management and control; 2. Occupations and services for the organization of activities; 3. Occupations and services related to marketing activities.

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Item number	Category of occupations and services	Production processes accompanying the provision of PSs	Types of work and services performed
3	Augmented occupations and services	Supporting processes are a set of production processes aimed at creating conditions for the production of PSs	1. Resource-supporting occupations and services; 2. Occupations and services to ensure the sanitary regime; 3. Occupations and services for the maintenance of equipment; 4. Occupations and services for the distribution of goods; 5. Occupations and services for loading and transporting goods.
4	Developing occupations and services	Main processes are a set of production processes aimed at preserving the consumer properties of goods within a pharmacy organization	1. Occupations and services related to the purchase of goods; 2. Occupations and services related to the acceptance of goods; 3. Occupations and services related to manufacturing medicinal products; 4. Occupations and services related to the storage of goods
5	Practical (true) services	Basic processes are a set of production processes aimed at the interaction of a pharmaceutical specialist with an PA consumer	1. Services related to the sale of goods; 2. Services related to the release of goods; 3. Information and consulting services; 4. Services that ensure customer loyalty.

According to the analysis of the scientific literature, it has been established that the PS is a structural unit of the pharmaceutical market, it has the properties of both the product and the service itself. The PSs system operates within the pharmaceutical market regulated by the state and includes pharmaceutical manufacturers, intermediaries for the provision of PSs (wholesale and retail organizations), consumers of goods and PSs. The relationship between these elements is carried out through goods-money relations and information interaction. As a result, PSs have a market value, i.e. they acquire the properties of a product and can act as an object of market relations [10]. In addition, this type of service, inherent in the service sector of the population, is the result of the professional activities of pharmaceutical specialists. It is quite natural that the main requirement for the conditions for providing PSs is the security of their provision [2].

When analyzing the economic characteristics of PSы, Russian researchers emphasize their high social priority and importance in maintaining and preserving people's health [2, 10, 28, 45, 46].

In our opinion, the study of the PSs essence from the standpoint of service management and service marketing is of considerable interest. First of all, it should be noted that PSs reinforce the very purpose of pharmaceutical products and form the population's need for the corresponding marketable types of pharmaceutical produce. It is no coincidence that such foreign scientists as G. Assel and F. Kotler, who consider the category of goods as a complex of tangible properties and intangible advantages, designed to meet the needs of consumers, emphasized the inextricable link between goods and services that support them. The marketing strategy put forward by them served as the basis for the conceptual model of a pharmaceutical product developed by the authors (Fig. 3).

The developed model consists of 3 internal inextricably linked blocks, which are integral, mandatory and interacting components of a pharmaceutical product, complementing each other and generally forming a full-fledged product of labor that is in demand among PA consumers.

The first block contains the basis of the corresponding product (core) and is an intangible component of a given pharmaceutical product, which determines the presence of the merits for which the product is purchased by the buyer and the benefits that will be obtained as a result of its use. The intangible components of the product encourage the buyer to purchase it to meet existing needs and expectations.

The second block contains the same product in real performance (a physical product), which is a tangible component of a pharmaceutical product. It has certain properties and characteristics that provide an opportunity for an appropriate description of the specified product. At the same time, tangible properties of a product should include not only physical characteristics (taste, smell, color), but also the level of its quality, a type of a dosage form, a number of units, comfort and ease of use of a consumer packaging, a name of a manufacturer, brand (brand), etc. The tangible components reinforce the buyer's position when choosing a product.

The third block contains the same product with reinforcement (an augmented product) – a set of PSs accompanying the product. This component of a pharmaceutical product implies accompanying the sale of the product with benefits for the buyer (both before and during the sale, as well as after it). This component, as a rule, in-

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cludes the PSs, accompanying a pharmaceutical product throughout the entire life cycle and contributing to the preservation of its consumer properties in the process of bringing it to the consumer to provide the greatest effect, as well as service and after-sales services.

The above-listed components of the internal blocks of a pharmaceutical product are equally involved in the formation of its consumer properties, which further determines the use value of the specified product and the degree of its compliance with consumer expectations and preferences, which are taken into account by the buyer when making a purchase [53].

For a proper understanding of the considered content of PSs and their consumer usefulness, the concept of multi-altributivity of goods, which is widely used in marketing services, was additionally used. With this concept in mind, PSs can be viewed as a set of attributes, i.e. the benefits that the consumer is looking for. However, it should it should be also taken into account that attributes (separate indispensable and integral components of a service), in the eyes of a PSs consumer, have unequal significance. In different services there are different sets of attributes, respectively, the consumer's assessments in relation to the presence and expression of this or that attribute in services are also different. Knowing the relative (subjective) significance of the attributes allows a pharmaceutical organization to develop a segmentation strategy aimed at providing a wide range of PSs with an appropriate level of service.

From the standpoint of the multi-attribute concept, in the aggregate, the content of the PSs includes three main components:

- activities to dispense and preserve the quality of pharmaceutical products through compliance with the rules and regulations for their circulation (a core service);
- organizational, managerial and other activities aimed at creating conditions for the production of core (necessary) services;
- activities related to pharmaceutical services aimed at creating concomitant conditions that have a beneficial effect on the level of customer satisfaction and the formation of their loyalty (an augmented service).

It should be noted that the set of core services corresponds to the PSs functional use of a certain class of goods, ensures their efficiency and safety. A pharmaceutical organization, by the specifics of its activities (provision of special storage conditions, a system of protection against poor-quality goods, maintenance of a mandatory range), guarantees the quality and safety of all received pharmaceutical products. Additional services form secondary usefulness and ensure the satisfaction of the consumer's needs for some secondary qualities of the product (attributes of aesthetic, emotional and other kinds of nature). A necessary service lies in the proper organization of the work of the retail entity, strategic planning of its activities to achieve the stated mission. The augmented services are not directly related to the "core" service and represent an important element of the service activities of the pharmacy.

Based on traditional concepts and the "4 negations" rule inherent in the paradigm of service activities, the decisive and most characteristic difference between a service and a product is the possession of at least the following four specific features: immateriality (intangibility), non-persistence, inseparability from the source, and inconstancy of quality. In addition, the key features of the difference between a service and a product should also include the continuity of production and consumption processes, fragility, lack of ownership, heterogeneity, complexity of assessment, a consumer involvement in the service process, etc. However, these characteristics cannot be considered universal for all types of services.

Guided by the above-listed specific key features of the difference between a service and a product, in the course of the study, the most characteristic features that make up the essence of PSs, were identified, generalized and grouped into the following nine groups (Table 3).

1. An attitude towards the product (a monetary-dependent service) means that the PSs market occupies an intermediate position between the market for materially-tangible goods and the "pure services". First of all, this circumstance is associated with a high content of the main commercial component of PSs – a pharmaceutical product. The implementation of this type of service is aimed at preserving or changing the consumer properties of a material product that increase its use value. In practice, the produced PSs are "embedded" and in a certain way "substantiated" in the objects that the services themselves are aimed at (in the pharmaceutical product). Accordingly, the majority of PSs can be sold to PA consumers only in conjunction with a pharmaceutical product in the process of a pharmaceutical service and do not represent any value of its own to the consumer. This circumstance is confirmed in the works by a number of authors. So, P.A. Lisovsky highlights a PS of the material and informational character, the essence of which is to provide accurate information about the drug, excluding its misuse, and the result of the provision of such a service is the purchase of these MPs [11]. According to T.V. Zernova a PS is also product-containing and includes 2 basic components: tangible and intangible ones [28].

2. An attitude towards the consumer means that the character of the PSs is determined by the essence of the consumer. PSs can reliably be classified as personal services (when providing them to the population), and as public (institutional) services when providing them to other organizations to be used in the production of the services their own.

3. An attitude towards society (socially significant services) means that PSs are provided to the population in order to improve their living conditions, preserve and

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maintain their healths. The effectiveness of the PSs provision directly affects the effectiveness of treatment and prevention of diseases, improving the quality of life of the population.

4. An attitude towards perception (the services intangible by the consumer) means that, like other types of services, PSs are intangible for the consumer, in most cases, they can only be perceived at the mental level together with a pharmaceutical product. The consumer is often not aware of the labor operations taking place within the pharmaceutical organization, and therefore cannot feel the process of providing PSs during the pre-sale preparation of goods and the preservation of their consumer properties. However, when providing a number of PSs, a tangible component can also be distinguished. It has a "useful effect" for the consumer and can be perceived by him when making a purchase and embodied in a purchased product (a direct delivery of goods, information and consulting assistance, additional services).

5. An attitude towards the contractor (professional services are inseparable from the contractor) means that PSs belong to the category of professional services and cannot be provided to the consumer without a participation of a specialist who has the appropriate professional training and level of qualifications. The exception is the PSs which can be provided without a direct participation of a specialist , with the help of technology (including information and computer technologies).

6. An attitude towards quality (conditionally constant quality services) means that the quality of PSs on the whole depends on the personality of the contractor and on the circumstances in which they appear to be. In this regard, the definition and measurement of the quality of services is difficult. However, taking into account the current trends and a global consumer orientation, more and more attention is paid to the unification of the quality of the provision (standardization) of services. The quality level of PSS is generally determined by the system of state regulation of pharmaceutical activities. However, in the authors' opinion, to a greater extent, the quality of PSs depends on the level of the QMS of the pharmaceutical organization and the internal control mechanism for its proper PSs functioning. The application of standards and norms has a beneficial effect on the consistency of the PSs quality, since it allows us to establish uniform requirements for the level of their quality and the technology of their provision. In order to reduce the variability of the quality of PSs on the basis of a thorough analysis of their parameters for retailers, it is advisable to develop not only SOPs for production processes, but also the Standard for pharmaceutical services as a rational model of the public service quality.

7. An attitude to the degree of interaction with the consumer in the process of providing services depends on the degree of a direct participation of the consumer in the production of services. There are two categories of PSs:

- the services of internal activities of a pharmacy organization (a non-contact type of service) include various labor actions of personnel aimed at the formation and preservation of consumer characteristics of goods within a pharmacy organization. The provision of such services does not require a direct presence of the consumer or requires his participation only partially.
- the services of interaction with the consumer (a contact type of services) include a number of labor actions of personnel that require the mandatory presence of the consumer and are carried out at the time of distribution (sale) of pharmaceutical goods, provision of information and consulting support, etc.

8. An attitude towards the duration of exposure as a temporary factor means that since pharmaceutical products are classified as non-durable goods, they are consumed at once or in several doses. In this regard, for the consumer, PSs are fragile services, and the frequency of their provision directly depends on the duration of the benefits obtained (for example, the therapeutic effect of the drug).

9. An attitude towards the production processes and consumption of services means that, according to most researchers, the PSs are characterized by the continuity of these processes, although some authors tend to consider the production and consumption of PSs separated both in time and space [44]. In the authors'opinion, it should be borne in mind that PSs and the production activity that facilitates their provision, are inseparable from each other, since they are embodied in the pharmaceutical product itself and are preserved in the "goods/works/services" complex. In this case, the process of converting the work performed into a service and the direct implementation of such a service occurs directly at the time of the sale of a pharmaceutical product to the consumer.

When considering the PSs subject area, it is advisable to highlight the following structural elements:

- a consumer (the object that the service itself is aimed at);
- a pharmaceutical specialist (a contractor, a service subject);
- relationships between the consumer and the pharmaceutical specialist (service psychology);
- a pharmaceutical product (a monetary component, a service carrier);
- the degree of satisfaction of the consumer's needs and expectations (a result of the service).

The structure and content of PSs are schematically shown in Fig. 4.

3. Development of nomenclature and types of PSS

In domestic pharmaceutical science, a number of authors have repeatedly attempted to classify PSs according to various criteria, but so far, the country has not developed a holistic approach to structuring, typology and classification of PSs.

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Due to the specificity and multidirectionality of PSs, the development of their rigorous scientific classification is rather difficult. Meanwhile, the need for scientific substantiation and development of a unified PSs nomenclature is caused both by the need for their certification and standardization, as well as forecasting demand and accounting for the implementation of services provided.

For a scientific substantiation of the PSs classification, domestic scientists use the general principles and approaches practised in the economy of the service industry. One of the most common variants of such an approach is the differentiation of PSs into basic ones aimed at the formation of the properties of goods, and augmented services – the ones that contribute to increasing consumer loyalty and attracting new customers [54].

According to the scientific classification suggested by E.A. Fedina, all the services provided by pharmacy organizations are divided into internal (carried out by specialists of a pharmacy of a medical organization to departments and divisions of this organization) and external (provided to the population and third-party organizations). In addition, in the studies by E.A. Fedina, the analyzed and systematized PSs are provided to pharmacies visitors. The author considers 3 classes of PSs: informational, consulting, materialized (associated with a direct release of a pharmaceutical product). At the same time, the author distinguishes services with a delayed purchase of pharmaceutical products of interest (the information about them and their purchase are carried out on different days, or not purchased at all) and services with a simultaneous purchase of the chosen pharmaceutical products [2].

P.A. Lisovskiy classifies PSs according to several criteria: by the nature of the service (informational and material-informational), the financing entity (commercial and budget-dependent), the degree of contact with a representative (a high and low degree of contact), depending on the consumer of the service (intermediate and final) [11].

E.S. Zvereva allocates PAs sale services (dispensing) and other kinds of services carried out within the framework of practical pharmaceutical activities (for example, manufacturing and packaging of drugs, dispensing drugs and PAs, reference and information services on the availability of drugs, providing advice, etc.) [55].

The PSs typology proposed by L.N. Geller, contains 12 main types of PSs, distinguished by six different characteristics. The list of the main types of PSs includes personal and public services consumed by the population and the organization, by market and non-market, basic and augmented, service ones, medical and health-improving services, as well as PSs with low and high degrees of a consumer participation in their production [45].

L.V. Moshkova et al. substantiated a simple (indivisible) PS (for example, providing information on the availability of a medicinal product), a complex PS, which is a combination of a complex of simple services (for example, dispensing an extemporal medicinal product by medical prescription = a drug manufacturing service and a service for dispensing a medicinal product) and a complex PS combining simple PSs of different kinds (for example, information on the availability of drugs in the pharmacy and dispensing of the requested drug) [54].

The PSs nomenclature was supplemented by A.M. Gosudarev in accordance with the types of pharmaceutical activities. Herewith, PSs are grouped by types: retail sales of medicinal preparations, dispensing drugs by free of charge and preferential medical prescriptions, dispensing drugs for medical organizations, manufacturing drugs, selling drugs by orders, realization of medicinal preparations by order, according to the principle of cumulative discounts, realization of medicinal preparations according to the principle of discounts for disadvantaged population, realization of medicinal preparations via the Internet [9].

K.A. Livshits allocates the actual service for the sale of pharmaceutical goods and the accompanying service for goods information support. According to the author, a pharmaceutical service acts as an integrated product in the market, combining a set of services for the sale of a pharmaceutical product and providing the consumer with information on the properties of the product. This information is comprehensive for his level of competence [10].

A list of targeted services that can be provided to the consumers in the field of occupational pathology has been formed by N.O. Karabintseva et al. This list includes the services for the sale of medicinal preparations, marketing, pharmacoeconomic, information and consulting services, sanitary and educational (educational) services, valeological, preventive and primary medical services. The authors also highlighted the services of pharmaceutical care, which include pharmaceutical consultations, control over the use of drugs, maintaining individual patient records, etc. The authors note that the provision of all these types of services is impossible without organizational activities, which are defined as an organizational service [17].

The works by a number of authors are focused on improving the quality of pharmaceutical services. Therefore, the essence of PSs has been considered by R.G. Dyachenko from the position of the QMS and a detailed list of pharmaceutical works and services has been developed to be included in the SOP, depending on the PSs functions performed by pharmacy organizations [13]. The main stages of medicinal preparations and PAs circulation in the pharmacy organization of the grouping of pharmaceutical works and services in the context of the performed PSs functions, proposed by V.N. Mikhailova et al., have been sufficiently detailed [47].

From the above mentioned examples it can be concluded that in the works by most authors, a pharmaceutical informative and consulting service is highlighted, it is present in all PSs classifications and is one of the nec-

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essary services in PAs dispensing. In many classifications there are augmented services focused on increasing competitiveness, attracting buyers and promoting sales. The existing classifications are based on the principle of allocation to a separate category of PSs associated with the sale and delivery of pharmaceutical products directly to consumers [13, 44, 46].

In the course of the study, the officially existing classification systems for pharmaceutical jobs and pharmaceutical services, have been studied. Currently, in our country, at the legislative level, the List of the works performed and the services provided, which form the profile of pharmaceutical activities, has been approved.[2] This list only enumerates the types of work and services in the field of medicinal preparations (MPs) circulation for the medical use, without their corresponding detailing, and includes wholesale trade, their storage, transportation, retail trade, dispensing, manufacturing. There is no such detailing in the current codes of pharmaceutical activities of the all-Russian classifiers (Russian Classification of Products by Economic Activities 2 и Russian National Classifier of Types of Economic Activity 2), which provide services for the wholesale trade of pharmaceutical products (code 46.46.1) and services for the retail trade of MPs in specialized stores (pharmacies) (code 47.73.1). The "Q" code (health and social services) presented in these classifiers of economic activities, does not contain the required PSs characteristics either.

The current "Nomenclature of works and services in health care" contains five main sections: simple medical services (section A), complicated and complex medical services (sections B, C), manipulations, research, procedures and works in health care (section D), medical services (section F). At the same time, PSs are missing in the nomenclature of medical services (sections A, B, C, F). Section D of the nomenclature "Manipulation, research, procedures and work in health care" sufficiently structures different types of work in the health care system. However, to a greater extent, the specified list of works is focused on the activities of medical organizations and their departments.

Code "D 08" of the specified list includes certain types of pharmaceutical occupations: pharmaceutical occupations in medical institutions (organizations), an occupation of a clinical pharmacologist, an occupation in the wholesale trade of MPs and the occupations related to the release (sale) of MPs in pharmacies.

To a certain extent, code "D 08.4" details the list of pharmaceutical occupations for retailers and includes the following:

- sale of officinal medicines to the population by medical prescriptions and without them (D 08.04.01);
- manufacturing MPs by medical prescriptions and requirements of healthcare institutions (organizations) and manufacturing intrapharmacy preparations (D 08.04.02);
- packaging of manufactured medicinal products and plant raw materials (D 08.04.03);
- dispensing medicines to the population (D 08.04.04);
- consulting patients by pharmacists (D 08.04.05);
- consulting medical specialists of health care institutions, education, social security and other persons, by pharmacists (D 08.04.06);
- organization of MPs storage and products medical reliese in pharmacies (D 08.04.07);
- ensuring control over compliance with the rules of storage of medicines and medicinal products in pharmacies (D 08.04.08);
- work on the registration, storage and distribution of narcotic drugs, psychotropic substances, toxic and superpotent substances in pharmacies (D 08.04.09).

However, the list presented is far from complete, it lacks such important types of pharmaceutical occupations as procurement and organizational and managerial activities, control and supervision, statistical types of work, etc.

The foregoing indicates that the current domestic pharmaceutical practice does not always take into account the entire breadth and diversity of the PSs production area, which, in turn, negatively affects the level and quality of pharmaceutical aid provided to the population.

In this regard, the American Model of The Pharmacy Practice Activity Classification (PhPAC) adopted by the American Pharmacists Association (APhA) and approved by the International Pharmaceutical Federation (IPF), is of particular interest. The PPAC classification includes groupings of codes for the types of activities of pharmacy practice, which, in turn, are detailed into a number of subgroups (Table 4)[3].

Taking into account clinical, economic and social positions, not only a classification of all types of pharmaceutical practice activities is provided in the presented model. There is also a new approach to the description and registration of the activities of pharmaceutical specialists, aimed at such an activity as the provision of PA, is demonstrated. The use of the classification made up using generally accepted terminology, contributes to obtaining comparable data for research in various directions. As a result, pharmacists can occupy a variety of positions in the pharmaceutical industry: in administrative and regulatory bodies, professional associations, the public health system, educational institutions, when they are directly involved in the provision of PA as colleagues and partners, ranging from traditional distribution of pharmaceutical products to direct providing services to the patient.

It should be noted that although the American model of PhPAC opens up a wide scope for pharmaceutical

[2] Decree of the Government of the Russian Federation of December 22, 2011 N 1081 "On licensing of pharmaceutical activities"

[3] Development of pharmaceutical practice: focus on the patient. WHO; MFF The Hague, The Netherlands, 2006: 110 p.

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specialists in the field of clinical pharmacy and clinical practice, providing for their participation as full members of the medical team, due to the specifics of professional training, this area of activity is still not quite accessible to Russian pharmaceutical specialists.

Providing qualified PA at all levels of the healthcare system, counseling, informing and monitoring drug therapy, as well as the technical aspects of providing PSs, require from pharmacists not only a thorough knowledge of pharmaceutical products, but also expert knowledge of therapy, a deep understanding of the pathogenesis of diseases and their symptoms [3].

In the authors' opinion, a set of pharmaceutical specialists and services produced by a specific retail entity, is formed taking into account its production activities and PSs operations performed. This circumstance determines the expediency of systematization and further standardization of PSs from the standpoint of the QMS, which allows to achieve the preservation of stability of quality parameters and characteristics of goods, as well as to increase the level of quality of providing the population with PSs [56].

Taking into consideration the fact that the production process is the basis of the activities of any enterprise, and also the activities of a retail entity as a sequential and cyclically repeated implementation of a set of interrelated production processes aimed at meeting the needs and expectations of PA consumers, the authors based the proposed classification model using the process approach. A process-oriented organization provides a continuous cycle of production of services within itself, as well as a well-functioning mechanism for controlling their quality. It is the application of the process approach that makes it possible to take into account the focus on the target tasks of the enterprise and the receipt of final results, as well as the interest of each contractor in improving the quality of the services provided [57].

The process of providing PSs in a retail entity is a closed and continuous cycle, accompanied by the transformation of input data (resources) into output ones – a finished product (Fig. 5).

The content of the production activity of a retail entity necessarily includes the following components:

- an expedient activity (labor) is carried out in the PSs system of operating production processes and contains a certain set of works;
- a subject of labor (a pharmaceutical product) is the object which the activity is aimed at;
- means of labor (a set of resources) are the means that can be used as instruments of influence in the process of transforming goods, adapting them to meet the needs of the consumer;
- a product of a production activity (a transformed pharmaceutical product) is a material benefit that meets the needs and expectations of PA consumers.

The model presented in the figure, makes it possible for us to conclude that the main object of influence in the production process of the PSs complex is a pharmaceutical product intended for the distribution (sale) to ultimate-users of PA. The production of PSs is accompanied by the transformation of the original form of a pharmaceutical product into a more complex (integrated) one, which can meet the needs and expectations of consumers to the full ex pharmaceutical tent. The process of transformation of a product is active and is the result of PSs integration of a system of interrelated production processes that add a consumer value to the original product and ultimately form a complex of PSs produced.

To transform pharmaceutical products in accordance with the requirements and expectations of PA consumers, as well as taking into account the established norms of the current legislation -—to the circulation of pharmaceutical products, it is required to produce not one specific PSs, but a whole complex containing a certain set of pharmaceutical works and services.

Thus, the result of the ongoing production processes is the formation of an integrated form of a pharmaceutical product containing a material carrier (a pharmaceutical product) and a certain set of occupations and services that increase its use value.

From the point of view of the QMS, the process of production and providing the population with PSs means the distribution of responsibilities among personnel, the implementation of an appropriate amount of work and a proper management (managing activities, planning, analysis of quality criteria for the services provided, control and optimization).

Accordingly, the content of each individual production process, the quality of its implementation and the effectiveness of the QMS functioning at the institutional level, have a decisive impact on the formation of the PSs quality and, consequently, on the procedure for providing PA itself.

Considering the forgoing, the production activity of a retail entity can be represented using the "pharmaceutical goods – pharmaceutical works – pharmaceutical services" complex, i.e. the transformation technology consists in adding the desired value to the original product through the use of various types of occupations and services.

As a result, depending on the nomenclature group of pharmaceutical goods, the specifics of logistics, a merchandise flow, price setting, acceptable storage conditions, and release conditions, there is a natural typology of the production activities of a retail entity to provide PSs. The whole complex of PSs, carried out by the subject of a retail trade, forms a typical variety of production processes of five consecutive provision levels (Table 5).

It should be noted that occupations and services of 1–4 levels (of strategic importance, organizational and managerial, augmented, developing occupations and services) have value only within a pharmaceutical orga-

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nization and represent a hidden range of services, intangible by the consumer. The provision of such works and services does not require the presence of a consumer, or requires only an insignificant extent. In turn, the provision of practical (true) services requires the mandatory presence of the consumer and is carried out directly at the time of the release (sale) of pharmaceutical goods, i.e. at the time of the transaction: purchase and sale, provision of information, consulting assistance, etc. The services of the fifth level are in demand by the consumer most of all and constitute the visible spectrum of the PSs.

Each of the listed levels of pharmaceutical occupations and services, is characterized by the performance of strictly defined labor actions, PSs and operations that contribute to the formation of a more detailed nomenclature of pharmaceutical occupations and services for retailers.

Thus, the process of PSs production is not homogeneous; in their performance, two successive stages can be distinguished. The first stage of providing PSs is carried out within a pharmaceutical organization itself, without contact with the consumer of the PA, by translating the labor actions of the personnel into a pharmaceutical product in order to preserve its consumer properties. On the other hand, for the second stage of PSs provision, a close contact with the PA consumer is required, accompanied by a high degree of individualization and a targeted approach in accordance with the requirements of the consumer.

4. Development of methodological approaches to optimizing the quality of PSs

A significant and relevant trend in the research of scientists from different countries is the study and assessment of the quality of PSs. The aim of such studies is to develop new and improve existing approaches aimed at optimizing the quality of PSs and, as a consequence, PA on the whole. The problem of delivering PSS of proper quality is becoming increasingly important. First of all, this circumstance is due to the dual socio-economic meaning inherent in PSs. On the one hand, a PS is one of the main tools for providing PA to the population, since the PSs implementation is expressed in meeting the main social need of a person – health maintaining, and therefore, the process of providing PSs requires adherence to certain ethical norms and rules. On the other hand, the provision of PSs refers to the sphere of public service and represents the sphere of production and economic activities of a pharmaceutical organization aimed at increasing the degree of consumer loyalty, obtaining economic benefits (profits) and the formation of certain competitive advantages.

According to the literature data, the studies related to the research and assessment of the customer satisfaction level, are currently quite relevant. Taking into account the client-oriented approach and a focus on the buyer, the level of customer satisfaction can be positioned as an indicator of a pharmaceutical organization functioning on the whole. It also demonstrates the degree to which the quality of the services provided meets the expectations of consumers. In this regard, consumer satisfaction is becoming an increasingly important indicator of the quality of any service type (including pharmaceutical) [59–61].

The authors from various countries (South Africa, Brazil, Ethiopia, USA, UK, Italy, Germany, Belgium, Denmark) show that customer satisfaction is an integral part of the PSs quality. Satisfaction influences adherence to treatment and loyalty to pharmacy organizations. In this case, the patient constitutes the core of PSs and the relationship with him includes not only technical, informational and communicative components, but also emotional aspects. The authors noted that the range of PSs is gradually expanding, the provision of this type of services goes beyond the traditional drug provision and becomes more and more focused on the patient. To achieve the best result and improve the patient's quality of life, an integrated approach and joint activities of pharmaceutical and medical professionals are required [61–66].

In Russian pharmacy, the administrative matters of PSs quality management at different stages of drug circulation are reflected, to one degree or another, in the works by E.A. Fedina, R.G. Dyachenko, P.A. Lisovsky, L.N. Geller, A.M. Gosudarev, S.V. Kononova, L.V. Moshkova, S.A. Smirnova and I.V. Kosova, K.A. Livshits and other authors [2, 9, 11, 13, 23, 44, 45]. According to K.A. Livshits, the PSs quality of is determined by the quality of its two components: the quality of the drug and the quality of information about the drug. The service quality is considered by E.A. Fedina as a set of characteristics of a service that determine its possibility to meet the established or assumed needs of a person. Some authors noted that the effectiveness of the provision and the quality of PSs depends on the person who provides it, where, how and when it was provided. In other words, the quality of the service largely depends on the personality of the contractor and the circumstances of its operation. Since PSs refer to social types of services and contribute to the maintenance and preservation of human health, for this type of service the criterion of "safety" is of great importance. The security of PSs provision is a set of requirements for the quality of professional actions of a pharmaceutical specialist and must be ensured without fail during its provision [2, 3]. Since the process of providing PSs to the population is a basic component of PA, the optimization of the PA management strategy dictates the need to achieve consistency in the quality of PSs and conduct their standardization.

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According to the literature data, as well as taking into account the modern realities of the current legislation, it has been established that an important role in ensuring the standard quality of pharmaceutical specialists and services, is assigned to the introduction of the QMS in the pharmaceutical industry, including the subjects of retail trade. The analysis of the literature sources indicates that the implementation and PSs organization of the QMS is a key tool in managing the quality of services provided and improving the QMS [67–70]. In our country, as in a number of other countries, national standards of Good Pharmacy Practice have been developed and are successfully applied on the basis of the QMS. Despite the fact that the activities of retail trade entities are regulated by the state, the established requirements for the implementation of pharmaceutical activities do not cover the entire range of activities of pharmacy organizations. Herewith, the need to form competitive advantages requires constant improvement of activities and an increase in the quality of services provided to the population. The experience of implementing the QMS shows that as a result, it is possible to identify weak links in the execution of individual production processes, determine the insufficient efficiency of certain relationships and direct resources to improve the quality of products and services provided.

The use of the QMS and the process approach in pharmaceutical industries is being actively studied at the present time. The works by EV Nevolina are devoted to modeling the QMS in pharmacy organizations. The author has identified and documented the main business processes and proposed a universal QMS model that meets the requirements of GOST R ISO 9000 standards [71]. Dyachenko R.G. A list of pharmaceutical occupations and services was developed for their inclusion in the QMS and the development of SOPs, an indicative model was proposed for assessing the effectiveness of the implementation of the QMS in the work of retail pharmaceutical organizations [13]. The matters of quality management of pharmaceutical goods and services from the position of the organization's QMS, were considered by F.N. Bidarova. The author proposed and developed approaches to creating a QMS for testing analytical laboratories [12]. Taking into account modern trends, the research related to substantiation of the essence and development of methodological approaches to assessing the effectiveness of the QMS in pharmaceutical organizations, is gaining relevance [72–75].

Despite the active study of the matters of PSs quality optimization, there is currently no holistic approach to the PSs quality management. The matters of quality management of pharmaceutical services in pharmacy organizations have been also worked out insufficiently.

The fact that the implementation of PSs to the population combines a range of pharmaceutical occupations and services related to the preservation of consumer properties and the sale of pharmaceutical products, has been taken into consideration. Herewith, the quality of the PSs should be determined with due regard to all their components: the quality of the pharmaceutical product; the quality of information accompanying a pharmaceutical product; the quality of professional training of the service provider. Taking into account the active and long-term contact of the consumer with the pharmaceutical specialists when receiving the PSs, the socio-ethical components of the PSs quality should be also taken into account. All these factors have a direct impact on the consumer's loyalty and the degree of his satisfaction with the service of a particular pharmacy organization.

The main task of management in the service sector is to ensure the quality of services that meet customer expectations and increase the level of customer satisfaction. The basis of the criterion of customers' judgments about the quality of a service product is its compliance with existing expectations. If the perceived quality has exceeded expectations, then the consumer is satisfied with the service, otherwise, he will remain dissatisfied. Thus, a key tool in PSs quality management is, first of all, a comprehensive assessment of their quality, which involves identifying critical points and justifying the necessary measures to improve the service process, and this is the plan of our further research.

CONCLUSION

The review indicates the presence of a number of trends in the development of domestic research in the field of PSs provision, which generally contribute to an increase in the quality of PA provision to the population. However, the most important role in research is assigned to the study and assessment of the quality of PSs, the development of approaches to its optimization. As evidenced by the results of the analysis and generalization, the most successful activity in the provision of services in the field of drug circulation requires the implementation of a process approach and the implementation of the QMS. As a result of the research, the main trends in the study of activities for the provision of services in the field of drug circulation, have been characterized. The results of a comprehensive analysis of the category of "pharmaceutical service" are presented in the following way: the terminological content of the concept has been cleared up, the groups of features characterizing the economic and social essence of PSs have been substantiated, and the systematization of pharmaceutical occupations and services using a process approach, has been proposed.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

L.N. Geller, A.A. Skripko – critical analysis of the scientific review and its editing,
A.A. Klimenkova – content analysis of periodicals and review writing,
L.A. Gravchenko – collection of materials for the preparation of the scientific review,
E.A. Korzhavykh – terminology formulation and general editing of the scientific review.

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RESEARCH ARTICLE

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APPROACHES TO THE SELECTION OF EXCIPIENTS FOR DENTAL GEL WITH CETYLPYRIDINIUM CHLORIDE

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The aim of the study was to determine the excipients influence on the characteristics of gels with cetylpyridinium chloride and to select the dental gel formulation gelation agents promising for the development of dental gel compositions. Hereby, the properties of the active pharmaceutical ingredient, characteristics of the specific gelation agents, as well as their influence on stability, biopharmaceutical and application properties of gels, were taken into account.

Materials and methods. In this study, polymers with various gelation mechanisms were considered. Their compatibility with cetylpyridinium chloride as well as storing kinetic and colloid kinds of stability, pH of aqueous solutions, spreadability and textural properties, a penetration ability by the agar diffusion method, an osmotic activity and rheological properties of the gels, were examined. For a complex evaluation of gel compositions study results, a desirability function was used.

Results. Stable homogenous dental gels with cetylpyridinium chloride can be obtained by using 25% poloxamer 407 and 5.0% high molecular weight chitosan as the basis.

The addition of poloxamer 188 to high molecular weight chitosan gels can produce stable systems with improved textural characteristics as well as increase their osmotic activity. Agar and low molecular weight chitosan addition significantly decrease, whereas poloxamer 188 and various molecular weight polyethyleneglycol increase the osmotic activity of 25% poloxamer 407 gels which are also characterized by a high penetration ability.

Conclusion. A complex evaluation of biopharmaceutical, physicochemical and application properties of the gels made it possible to establish that combinations of poloxamer 407 with polyvinylpyrrolidone, agar, and low molecular weight chitosan, can be recommended as a base for a dental gel with cetylpyridinium chloride**.**

Keywords: cetylpyridinium chloride; dental gel; composition; excipients; biopharmaceutical properties; chitosan; poloxamer; desirability function

Abbreviations: PP – pharmaceutical preparation; DF – dosage form; CMC – carboxymethylcellulose; PVP – polyvinylpyrrolidone; PEG – polyethyleneglycol; FDA – Food and Drug Administration (U.S.)

ПОДХОДЫ К ВЫБОРУ ВСПОМОГАТЕЛЬНЫХ ВЕЩЕСТВ ДЛЯ ГЕЛЯ СТОМАТОЛОГИЧЕСКОГО С ЦЕТИЛПИРИДИНИЯ ХЛОРИДОМ

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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Цель. Изучение влияния вспомогательных веществ на свойства гелей с цетилпиридиния хлоридом и выбор гелеобразователей, перспективных для разработки состава геля стоматологического. При этом учитывали свойства действующего вещества, особенности конкретных гелеобразователей, а также их влияние на устойчивость, биофармацевтические и потребительские свойства гелей.

Материалы и методы. В исследовании рассматривали полимеры с различными механизмами гелеобразования. Изучали их совместимость с цетилпиридиния хлоридом, устойчивость гелей при хранении, кинетическую устойчивость и коллоидную стабильность, pH водных извлечений, намазываемость и текстурные свойства, проникающую способность методом диффузии в агар, осмотическую активность и реологические свойства гелей. Для комплексного анализа результатов исследований гелевых композиций использовали обобщённую функцию желательности.

Результаты. Устойчивые однородные гели стоматологические с цетилпиридиния хлоридом могут быть получены при использовании в качестве основы 25% полоксамера 407 и 5,0% хитозана высокомолекулярного. Введение в гели хитозана высокомолекулярного полоксамера 188 позволяет получать стабильные системы с улучшенными текстурными характеристиками, а также значительно увеличивает их осмотическую активность. Добавление агара, а также хитозана низкомолекулярного значительно уменьшает, а полоксамера 188 и полиэтиленгликолей разных молекулярных масс – увеличивает осмотическую активность гелей 25% полоксамера 407, которые характеризуются также и высокой проникающей способностью.

Заключение. По итогам комплексной оценки биофармацевтических, физико-химических и потребительских свойств гелей установлено, что в качестве основы для геля стоматологического с цетилпиридиния хлоридом могут быть рекомендованы комбинации полоксамера 407 с поливинилпирролидоном, агаром и хитозаном низкомолекулярным.

Ключевые слова: цетилпиридиния хлорид; гель стоматологический; состав; вспомогательные вещества; биофармацевтические свойства; хитозан; полоксамер; функция желательности

Список сокращений: ЛП – лекарственный препарат; ЛФ – лекарственная форма; КМЦ – карбоксиметилцеллюлоза; ПВП – поливинилпирролидон; ПЭГ – полиэтиленгликоль; FDA – Управление по санитарному надзору за качеством пищевых продуктов и медикаментов США / U.S. Food and Drug Administration

INTRODUCTION

Currently, oral cavity diseases are ones of the most common. Periodontal diseases can be distinguished among them. Periodontium is a complex of tissues that form supporting apparatus of teeth; frequent symptoms of periodontal diseases are inflammations, primarily or secondarily associated with the growth of the infectious microorganisms [1, 2].

Dental gels are ones of the dosage forms (DFs) used for the local treatment of periodontal diseases.

The pharmaceutical preparations (PPs) in such DFs, registered in Russia, belong to local anesthetic, antiseptic, antimicrobial, non-steroidal anti-inflammatory agents. In such compositions, antimicrobial components are metronidazole, chlorhexidine digluconate, cetylpyridinium chloride, cetalkonium chloride, benzalkonium chloride and their combinations [3].

Cetylpyridinium chloride is one of the most common antimicrobial preparations prescribed for inflammatory infections of the oral cavity [4]. Its therapeutic concentration safety was confirmed by the clinical data and proved by FDA [5–7].

Among the DFs with cetylpyridinium chloride for the oral cavity, sprays and lozenges are prevalent [3, 8, 9]. In dental gels, 0.1% cetylpyridinium chloride is presented only in combination with lidocaine hydrochloride in local anesthetic preparations prescribed for children in cutting of the teeth [3, 9].

Currently, a large number of studies on dental gels with synthetic and natural substances development, including those with antimicrobial properties, are known [10–20]. There are investigations on the study of local adhesive DFs with cetylpyridinium chloride [9, 21–25].

But to date, there is no submitted research on the development of dental gels which contains only cetylpyridinium chloride as an active pharmaceutical ingredient. Therefore, the development of such a PP is relevant [9].

DFs for the local use in the oral cavity contain from 0.05 to 0.50% cetylpyridinium chloride [3]. The developed gel will contain one active pharmaceutical ingredient. For this reason, the choice of the maximum therapeutic concentration (0.50%) for this DF is appropriate.

When developing a gel composition, the base choice which will affect the biopharmaceutical properties of the DF, including its penetration ability and osmotic activity, is very important. The prolongation of gel presence on mucosa can be achieved by including mucoadhesive polymers as well as their combinations, in the gel composition. It is important to consider this fact in the pharmaceutical development of this DF [26–29].

It is common knowledge that the characteristics of active pharmaceutical ingredients can impact the physicochemical and technological properties of a gel base. This fact is especially important for cetylpyridinium chloride, which, as a cationic compound, can affect the conformation of gelation agents molecules, sensitive to the presence of ions in the system [9, 27, 30].

The possibility of the chemical interaction between cetylpyridinium chloride and acids should be also taken into account. The problem of combining cetylpyridinium chloride and acidic compounds in one DF is complex and depends on the type of the DF, its aggregate state, the presence of stabilization agents, the method of substances introduction and, in particular, the degree of substitution of acid groups in cellulose derivatives gelling agents [9, 23, 25, 31].

Hence, the selection of excipients for dental gel base is a complex problem that should be solved by determining biopharmaceutical, physicochemical and technological characteristics of a gel base with the consideration of special aspects of this DF administration.

THE AIM of the study was to determine the excipients influence on the characteristics of gels with cetylpyridinium chloride, and to select the dental gel formulation gelation agents promising for the development of the dental gel composition.

The research objectives included the selection of compatible with cetylpyridinium chloride gelation agents and viscosity modifying agents, the selection of their concentrations, the investigation of physicochemical and biopharmaceutical properties of the gel compositions, their complex evaluation and the excipients selection for the dental gel.

MATERIALS AND METHODS

The cetylpyridinium chloride substance ("Diam", Russia) and the excipients – acid-soluble high molecular weight chitosan (ZAO «Bioprogress», Russia), aqua-soluble low molecular weight chitosan (ZAO «Bioprogress», Russia), polyvinylpyrrolidone (PVP) (Plasdone K 29/32, ISP Pharmaceuticals, Switzerland), agar (Agar 900, Qixiang, China), poloxamer 407 (Kolliphor P 407, BASF, Germany), poloxamer 188 (Kolliphor P 188, BASF, Germany), polyethyleneglycol 300 (PEG 300) (Polyethylenglycol 300, Merck, USA), polyethyleneglycol 6000 (PEG 6000) (NORCHEM-008, Russia), glycerine (NevaReaktiv®, Russia), carbomer (Carbopol™ 974 P NF, IMCD, Netherlands), sodium alginate (FOODALRA®, 500, FOODCHEM, Russia), iota carregeenan (Benvisco, USA), xanthan gum (NOW® FOODS, USA), methylcellulose (Methocel™A15, DOV cemical company limited, USA), carboxymethylcellulose (CMC) (Akucell® AF 2785, Akzo Nobel, Netherlands), acacia gum (Instantgum™ BA, CNI, France) – were used.

N-tris hydroxymethyl-aminomethane (trometamol) (NevaReaktiv®, Russia) was used to obtain carbomer gel and hydrochloric acid for chitosan gel (Lenreaktiv, Russia). A 10% Cetylpyridinium chloride aqueous solution was introduced in the gels.

The storing stability was examined by gels exposure infilled to the top orange glass jars (BTC-20-27,5-OS-1 type) at the room temperature for 6 months. The gel samples' appearance, homogeneity and consistency were determined.

A kinetic stability was studied by centrifugation of the gel samples at 6000 rpm for 15 min. The kinetic stability coefficient (H_k) was calculated by the formula (1):

$$H_k = \frac{H_1}{H_{tot}}, \quad (1)$$

where: H_1 is the height of the released fluid layer, H_{tot} is the total height of the gel layer [32].

The gel was considered kinetic stable at H_k=0.0.

The colloid stability was determined by centrifugation of the gel samples at 6000 rpm for 5 min after their freezing and thawing [33]. The colloid stability coefficient (K_c) was calculated as the ratio of the phase height released after centrifugation to the total height of the gel sample in the centrifuge tube. For the colloid stable gels, the value of K_c should be equal to 0.0.

The spreadability and textural properties were determined by estimation of the gel sample (0.5 g) distribution between the glass plates with a force of 0.5 kg [34].

The penetration ability was studied by the agar diffusion method according to the developed methods [20] based on the classic approach to the biopharmaceutical characteristics of semi-solid dosage forms [33].

The determination of the osmotic activity was carried out by the Kruvchinsky equilibrium dialysis method through a semipermeable membrane with a pore diameter of 12–14 kDa («Orange Scientific», Belgium) [35]. A 0.9% aqueous solution of Sodium chloride was used as a dialysis liquid.

The dynamic viscosity was examined on Brookfield DV-II+PRO programmable viscometer (Brookfield Engineering Laboratories, Inc., США) subject to OFS. 1.2.1.0015.15 "Viscosity" [36]. The sample viscosity in the range of shear rates from 10 to 200 s^{-1} and from 200 to 10 s^{-1} was determined. The torque values ranged from 30 to 80%.

For complex evaluations of gel compositions, a desirability function was used [37].

The results of parallel measurements were processed according to GPM 1.1.0013.15 "Statistical processing of the results of a chemical experiment" [36].

RESULTS AND DISCUSSIONS

Selection of compatible excipients

At the first stage, the compatibility of cetylpyridinium chloride with widely used in dental gels excipients, was studied [3, 10, 12–15, 17–19, 27]. The calculated amounts of a 10% aqueous solution of cetylpyridinium chloride were added to gels or excipients aqueous solutions until its 0.5% concentrations in the samples. The obtained systems were described immediately and after 30 days of storage. The mixtures of cetylpyridinium chloride with 1.0% carbomer, 2.0% iota carrageenan, 8.0% acacia gum, 2.0% methylcellulose, 4.0% carboxymethylcellulose, 6.0% high molecular weight chitosan, 4.0% low molecular weight chitosan, 20.0% poloxamer 407, 15.0% poloxamer 188, 1.0% agar, 3.0% xanthan gum, 1.0% sodium alginate, 20.0% PVP, 10.0% PEG 300, 5.0% PEG 6000 and 40.0 glycerol were studied.

It has been established that heterogeneous systems are formed at the addition of a cetylpyridinium chloride solution to carbomer, iota carrageenan, cellulose derivatives, xanthan gum gels, and acacia gum solutions. There were various kinds of a residue structure of these systems – from the white adhesive mass in carrageenan gels to a fine precipitate in methylcellulose gels.

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Homogeneous systems with no changes in the appearance were formed with chitosans, poloxamers, PVP, agar, PEG 400 and 6000 and glycerol. High molecular weight chitosan and poloxamer 407 were selected as a base gelation agent with the properties of their aqueous systems taken into account.

Poloxamer 407 is a non-ionic polymer of a synthetic origin. It is used as a gelation agent, a viscosity modifying agent, emulsifier and solubilizer (HLB value 18–23) in liquid and semi-solid DF technologies. Thermoreversible properties of poloxamer 407 are its technology features. Gelation mechanism is connected with a micelles formation and association, as the temperature rises [38, 39].

Chitosan is a chemically modified gelation agent of the natural origin with known antiseptics and wound healing activities. The characteristics of chitosan molecules depend on their molecular weight. Gelation mechanism is connected with ionization and changes in molecular conformation at the interaction with acid solutions [29, 40, 41].

Chitosans, poloxamers, agar, PVP and PEG of various molecular weight have ~~of~~ mucoadhesive properties which are developed differently [26, 28, 29, 39]. Mucoadhesive properties are the aim of a separate research in the framework of a dental gel with cetylpyridinium chloride pharmaceutical development, and are not considered in this work.

Excipients influence on gel with cetylpyridinium chloride characteristics

At the next stage, the working concentration of gelation agents was chosen. For this textural, biopharmaceutical and physicochemical properties of 20.0–30.0% poloxamer 407 and 2.0–6.0% high molecular weight chitosan gels were investigated [20]. According to their properties, complex evaluation gels of 25.0% poloxamer 407 and 5.0%, chitosan were chosen for a further study.

To develop a composition that meets the requirements for dental gels, taking into account the characteristics of their use and the properties of the active substance, combinations of the main gelation agents with compatible excipients were made up. Their stability, *pH*, penetration ability, osmotic activity, spreadability, and textural properties, as well as organoleptic characteristics, were studied. Fifteen compositions of dental gels were selected for the investigation (Table 1).

Appearance, a storing stability, kinetic and colloid stabilities were determined for gels compositions (Table 2).

It has been established, that compositions 2, 3 and 5 with high molecular weight chitosan as a base gelation agent, separated into two phases after 6 months of storage. The gels separation can be explained by the significant difference between the molecular weight of the base gelation agent – high molecular weight chitosan (200 kDa) and viscosity modifying agents – low molecular weight chitosan (1–30 kDa), PEG 300 and 6000 as well as the absence of synergism during gelation. The gels formation was due to simple physical molecules interweaving of high molecular weight chitosan and the excipients. This did not provide stability of the systems, and further on, the gels separated into two phases – the lower one contained mainly high molecular weight chitosan, and the upper lighter phase contained viscosity modifying agents.

At the same time, by combining high molecular weight chitosan with poloxamer 188 (ratio 5:10 and 4:20) and PVP (5.0 : 1.5), stable homogeneous systems were obtained. However, composition 6 showed a change in consistency towards its density. For these reasons, 2, 3, 5 and 6 gels were excluded from the further study.

The gels with a poloxamer 407 base did not show any changes in properties.

For stable compositions, a pH value, spreadability and textural properties were determined (Table 3).

Based on the analysis of the data obtained, it has been established that compositions 1 and 7 with high molecular weight chitosan and poloxamer 407 without viscosity modifying agents addition had equally low spreadability values. However, the textural properties of cationic high molecular weight chitosan with the addition of a non-ionic surfactant (poloxamer 188) forming different conformation micelles (compositions 4 and 15) were improved significantly. Sample 15 has the highest spreadability value of all the studied gels.

It ~~is~~ has been additionally noted that when spreading over the surface, this composition formed foam. At the same time, the addition of poloxamer 188, PVP, PEG 300 and 6000 as well as low molecular weight chitosan in poloxamer 407 based gels, has a slight effect on their spreadability. Hence, the introduction of excipients improving textural properties, is necessary for the main part of the obtained gels.

Poloxamer 407 based gels were characterized by a high penetration ability. Poloxamer 407 is known to be used as a penetrant in a semi-solid DF technology [38, 39]. To approximate the model to physiological conditions, the penetration ability was determined by thermostating the samples at 37°C. When choosing the experimental conditions, it was noted that the agar diffusion depth for poloxamer 407 gels at the room temperature was higher than at 37°C. The viscosity poloxamer 407 gels increasing at rising the temperature can explain this fact. Therefore, their agar diffusion was difficult.

When studying the penetrating ability of the gels based on chitosan, it was found out that the samples absorbed water and spread over the surface of the medium (compositions 1, 4, and 15). No diffusion into the agar can be explained by a high molecular weight of the base gelation agent.

The pH value of all examined samples is close to the physiological pH values of mixed saliva [42].

The results of the gels osmotic activity determinations are presented in Fig. 1.

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Table 1 – Compositions of gels with cetylpyridinium chloride

Composition number	Content,%											
	CPC	Chitosan h.	P 407	Chitosan l.	PVP	Agar 900	P 188	PEG 300	PEG 6000	Glycerol	10% HCl	Purified water
1	0.5	5.0	–	–	–	–	–	–	–	–	5.0	to 100.0
2	0.5	3.0	–	4.0	–	–	–	–	–	–	3.0	to 100.0
3	0.5	5.0	–	–	–	–	–	3.0	–	7.0	5.0	to 100.0
4	0.5	5.0	–	–	–	–	10.0	–	–	–	5.0	to 100.0
5	0.5	5.0	–	–	–	–	–	–	10.0	–	5.0	to 100.0
6	0.5	5.0	–	–	1.5	–	–	–	–	–	5.0	to 100.0
7	0.5	–	25.0	–	–	–	–	–	–	–	–	to 100.0
8	0.5	–	25.0	–	–	–	10.0	–	–	–	–	to 100.0
9	0.5	–	25.0	–	–	–	–	10.0	–	–	–	to 100.0
10	0.5	–	25.0	–	–	–	–	3.0	7.0	–	–	to 100.0
11	0.5	–	25.0	–	–	0.1	–	–	–	–	–	to 100.0
12	0.5	–	25.0	–	1.5	–	–	–	–	–	–	to 100.0
13	0.5	–	30.0	0.1	–	–	–	–	–	–	–	to 100.0
14	0.5	–	25.0	0.4	–	–	–	–	–	–	–	to 100.0
15	0.5	4,0	–	0.2	–	–	20.0	–	–	–	4,0	to 100.0

Note: CPC – cetylpyridinium chloride; chitosan h.– high molecular weight chitosan; P 407 – poloxamer 407; chitosan l.– low molecular weight chitosan; P 188 – poloxamer 188; 10% HCl – 10% hydrochloric acid solution

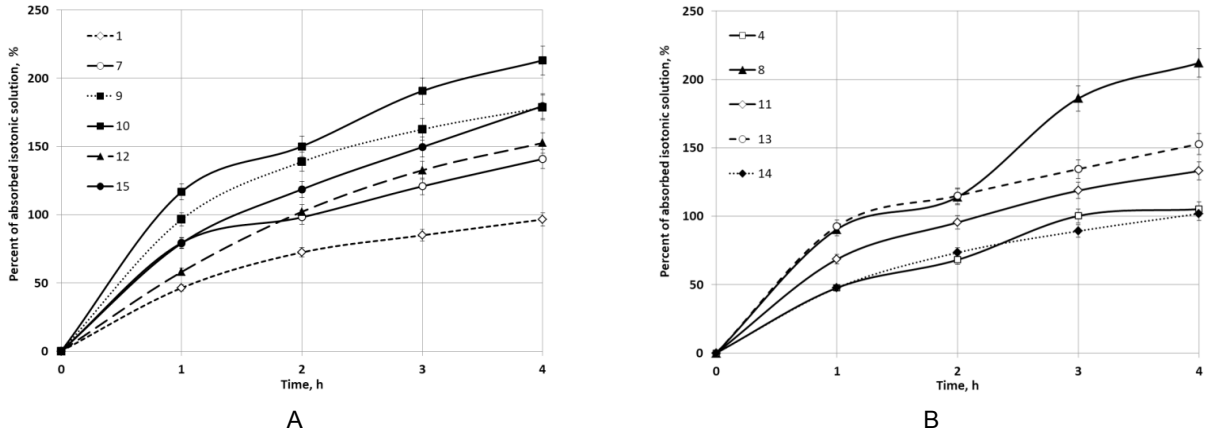

Figure 1 – Dependence of the absorbed isotonic solution mass fraction on the time of gel dialysis

Note: A – compositions 1, 7, 8, 10, 12 and 15; B – compositions 4, 8, 11, 13 and 14

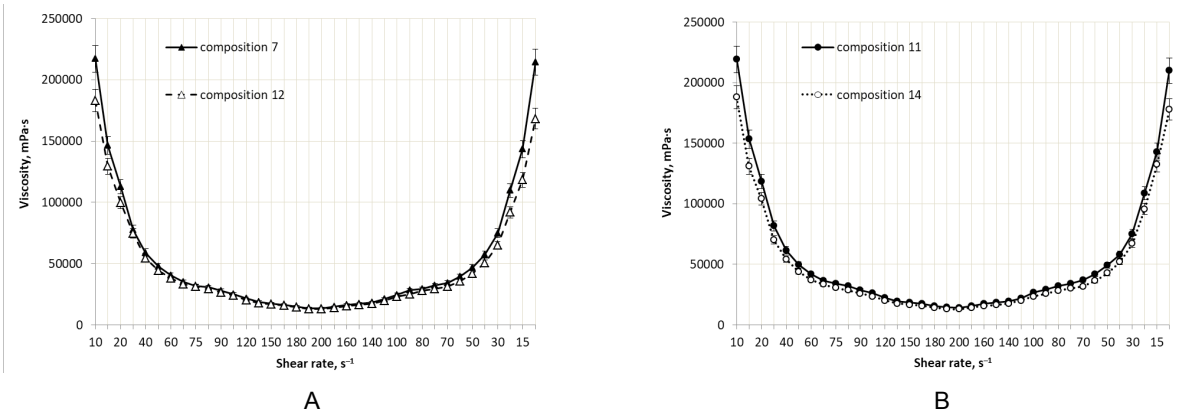

Figure 2 – Viscosity curves for compositions 7, 12 (A) and 11, 14 (B) at 20°C

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Table 2 – Stability of gel samples with cetylpyridinium chloride

Composition number	Samples appearance after receipt			Samples appearance after 6 month of storage		
	Appearance	H_k	K_c	Appearance	H_k	K_c
1	Slightly opalescent homogeneous dense yellowish non-flowing mass	0.0	0.0	No changes	0.0	0.0
2	Opalescent homogeneous dense light brown non-flowing mass	0.0	0.0	Separation into a clear liquid and strongly opalescent gel phases, approximately equal in volume	0.4	0.6
3	Opalescent homogeneous dense whitish flowing mass	0.0	0.0	Separation into a clear liquid (about 30% in volume) and strongly opalescent gel phases	0.3	0.5
4	Strongly opalescent yellowish flowing mass	0.0	0.0	No changes	0.0	0.0
5	Strongly opalescent dense yellowish flowing mass	0.0	0.0	Separation into a clear liquid and strongly opalescent gel phases, approximately equal in volume	0.3	0.4
6	Strongly opalescent dense yellowish non-flowing mass	0.0	0.0	Strongly opalescent jelly-like mass	0.0	0.0
7	clear homogeneous dense colorless non-flowing mass	0.0	0.0	No changes	0.0	0.0
8	Clear homogeneous dense colorless non-flowing mass	0.0	0.0	No changes	0.0	0.0
9	Clear homogeneous dense colorless non-flowing mass with foam on the surface	0.0	0.0	Clear homogeneous dense colorless non-flowing mass	0.0	0.0
10	Clear r homogeneous dense colorless non-flowing mass*	0.0	0.0	No changes	0.0	0.0
11	Slightly opalescent homogeneous dense colorless non-flowing mass	0.0	0.0	No changes	0.0	0.0
12	Strongly opalescent homogeneous dense colorless non-flowing mass*	0.0	0.0	No changes	0.0	0.0
13	Strongly opalescent homogeneous dense yellowish non-flowing mass	0.0	0.0	No changes	0.0	0.0
14	Opalescent homogeneous light brown non-flowing mass with a light texture	0.0	0.0	No changes	0.0	0.0
15	Slightly opalescent homogeneous dense yellowish non-flowing mass with air bubbles in volume and foam on the surface	0.0	0.0	No changes	0.0	0.0

Note: *Gel acquires a liquid consistency under cooling

Table 3 – Characteristics of gel samples with cetylpyridinium chloride

Parameter	Composition number										
	1	4	7	8	9	10	11	12	13	14	15
pH value of 5.0% aqueous gel solutions	5.45±0.05	5.30±0.04	6.46±0.05	6.22±0.05	6.14±0.04	6.26±0.05	6.36±0.04	6.16±0.05	5.20±0.05	5.54±0.05	5.34±0.05
Agar diffusion depth, mm	–	–	13.9±0.4	14.1±0.7	14.6±0.5	12.6±0.5	15.0±0.9	12.6±0.5	14.8±0.9	15.0±0.6	–
Spreadability, cm	3.3±0.3	3.8±0.4	2.7±0.3	2.6±0.2	3.1±0.3	2.6±0.4	3.1±0.2	3.0±0.3	2.9±0.3	3.0±0.4	5.0±0.3

Table 4 – Desirability function response variables

Desirability	Individual desirability functions	Desirability function response variables					
		d_1	d_2	d_3	d_4	d_5	d_6
«very good»	[0.80; 1.00]	6.80–7.40	0.0–5.0	20–40	4.0–5.0	The gel has a light texture, evenly distributed with little effort, completely remains on the distribution surface	Neutral
«good»	[0.63; 0.80]	6.20–6.79	5.1–10.0	41–60	3.0–3.9	The gel has a light texture, the distribution is slightly difficult, a small part of the gel remains on the spreading surface	Mild, easily corrected
«acceptable»	[0.37; 0.63]	5.60–6.19	10.1–15.0	61–80	2.0–2.9	The dense gel, evenly distributed with effort, most of the gel remains on the distribution surface	pronounced, correction is possible
«bad»	[0.20; 0.37]	5.00–5.59	15.1–20.0	81–100	1.0–1.9	The dense gel, distributed unevenly, while it may foam or form an inelastic film, which remains equally on both the distribution surface and the spreading surface	Very pronounced, the correction is difficult
«very bad»	[0.00; 0.20]	4.20–4.99	more than 20.0	more than 100	less than 1.0	The dense gel, the surface distribution is difficult, most of the gel remains on the spreading surface	Very pronounced, the correction is impossible

Table 5 – Generalized desirability coefficient values for dental gels

Composition number	1	4	7	8	9	10	11	12	13	14	15
D value	0.34	0.36	**0.60**	0.53	0.35	0.35	**0.62**	**0.68**	0.43	**0.55**	0.44

It has been established that samples 1, 4 and 14 with high molecular weight chitosan, its combination with poloxamer 188 (5:10) and a composition of poloxamer 407 and low molecular weight chitosan (25:0,4) have the lowest osmotic activity value (less than 50% per first exposition hour). At the same time, 20% of poloxamer 188 addition in chitosan gel significantly increased its osmotic activity.

The additions of agar (sample 11), as well as low molecular weight chitosan (sample 14) significantly reduce the osmotic activity of 25% poloxamer gels.

The osmotic activity increases with the increase of poloxamer 188 and 407 concentration (compositions 8 and 13). PEG 300 and 6000 (composition 10 and 9) introduction significantly increases the poloxamer 407 gels osmotic activity.

Meanwhile, sample 10 has the highest value of the osmotic activity: its mass increases more than twice for the first exposition hour.

Compositions complex evaluation for the selection of dental gel base with cetylpyridinium chloride

The following demands to the excipients selection were taken into account: the gel base should have a light spreadability and good textural properties, pH value close to the physiological condition, a low osmotic activity (to avoid the moisture loss by mucus membranes), a low penetration depth (to prevent the entry of local antiseptics into the bloodstream) and a neutral flavour [12–14, 27].

Harrington's generalized desirability function can provide a complex evaluation of gels compositions [37]. Taking into account the above-listed requirements for dental gels, the following responses of the desirability function have been selected:

1. d_1 – pH value of 5.0% aqueous gel solutions;
2. d_2 – agar diffusion depth, mm;
3. d_3 – osmotic activity per first exposition hour;
4. d_4 – spreadability, cm;
5. d_5 – textural properties;
6. d_6 – flavour.

The characteristics of desirability function response variables are presented in Table 4.

Generalized desirability coefficient (*D*) was calculated by the formula (2):

$$D = \sqrt[6]{d_1 \times d_2 \times d_3 \times d_4 \times d_5 \times d_6}, \quad (2)$$

The results of the determination are presented in Table 5.

Based on the analysis, it was established that composition 12 with poloxamer 407 and PVP has a high generalized desirability coefficient value.

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Meanwhile, compositions 7, 11 and 14 also had rather high *D* values. Their bases contain 25% poloxamer and its combinations with agar and low molecular weight chitosan. These gels can be used as a base with the introduction of excipients that correct their properties.

When choosing excipients, their effect on the structural and mechanical properties of gels is of great importance.

That's why at the next stage, rheological characteristics of compositions 7, 11 and 14 were determined. The viscosity curves are presented in Fig. 2.

It has been established that all studied samples had similar dynamic viscosity values in the range of shear rates from 30 to 200 s^{-1}. With an increase in the shear rate, the dynamic viscosity of all these compositions decreased, which makes it possible to attribute it to systems with a pseudoplastic type of flow. It should be noted that all examined samples restored the viscosity after unloading, which allows predicting the retention of the structural and mechanical properties of these systems during their production and packaging.

CONCLUSION

Cetylpyridinium chloride forms stable homogeneous systems with chitosans, poloxamers, PVP, agar, PEG 300 and 6000. When studying the properties of gels containing these substances in different combinations, their influence on the properties of the resulting compositions was determined. Hence, it was found that poloxamer 188 addition to the high molecular weight chitosan (20:4) can obtain stable systems, as well as improve their spreadability and textural properties, and increase their osmotic activity. At the same time, a further stabilization is required by combinations of high molecular weight chitosan with PEG 300 and 6000, low molecular weight chitosan (in the ratio 5:3, 5:10 and 3:4, respectively) to prevent their separation.

Poloxamer 407 based gels were characterized by a high penetration ability. The studied excipients (poloxamer 188, agar, low molecular weight chitosan, PVP, PEG 300 and 6000) do not significantly impact on these characteristics. Additions of agar, as well as low molecular weight chitosan, significantly reduce the osmotic activity of 25% poloxamer gels., while poloxamer 188 and PEG of various molecular weight increase it. It should be noted that poloxamer 407 gels require the introduction of excipients that improve their texture properties.

The complex evaluation of gels biopharmaceutical, physicochemical and application properties established that combinations of poloxamer 407 with polyvinylpyrrolidone, agar, and low molecular weight chitosan can be recommended as a base for a dental gel with cetylpyridinium chloride. These systems were characterized by the pseudoplastic type of flow and restored the viscosity after an applied load of shear rates in the studied range.

The final composition selection can be made after studying mucoadhesive and antimicrobial properties of the developed dental gels, which will be discussed in the further research.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

E.Yu. Zagorulko – determination of the aim and objectives of the study, planning of the experiments and interpretation of their results, selection of the desirability function responses and calculation of the generalized coefficient values, preparation of the article manuscript; A.S. Karavaeva – execution of the experimental work, statistical processing of the primary data, discussion of the research results.

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MULTISENSORY COLORIMETRIC ANALYSIS OF DRUGS DYDROGESTERONE, TROXERUTIN AND ADEMETIONINE USING BARCODES

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The aim of this study is to develop a universal, rapid and affordable method for the identification of dydrogesterone, troxerutin, and ademetionine in drugs by multisensor digital colorimetry using a unique two-dimensional code. The developed approach can be applied to rapid detection of counterfeit drugs at the preliminary stage of the analysis (before using more expensive specialized equipment).

Materials and methods. To implement the proposed approach, the substances of dydrogesterone ("Abbott Biologicals B.V.", Netherlands), troxerutin (JSC "Interfarma", Prague, Czech Republic) and ademetionine (LLC "Farmamed", Moscow, Russia), troxerutin capsules 300 mg (LLC "Pranafarm", Samara, Russia), lyophilisate for an intravenous solution and the intramuscular administration "Heptral"® (ademetionine) 400 mg ("Abbott Laboratories", GMBH, Germany), tablets "Duphaston"® (dydrogesterone) 10 mg ("Abbott Healthcare Products B.V.", Netherlands), were used. A multisensor colorimetry method has been implemented using the following set of 8 sensors (C_1–C_8): an intact solution – a 96% (v/v) aqueous ethanol solution – C_1; 1 mM alcoholic solution of anthraquinone green (CAS#4403-90-1) – C_2; a 0.2% aqueous solution of 3-methylbenzothiazolinone hydrazone (CAS#1128-67-2) – C_3; a 0.2% methyl orange aqueous solution (CAS#547-58-0) – C_4; a 1 mM alcoholic solution of sulforhodamine B (CAS#3520-42-1) – C_5; a 1 mM alcoholic solution of 1-hydroxypyrene (CAS#5315-79-7) – C_6; 1 mM alcoholic solution of allura red AC (CAS#25956-17-6) – C_7; a 1 mM aqueous solution of iron (III) chloride – C_8. Transparent flat-bottomed polypropylene plates with 96 cells, with a cell volume of 350 µl (Thermo Fischer Scientific, USA, cat. No. 430341) were used as a base for the chip. For obtaining raster images, an Epson Perfection 1670 office flatbed scanner (CCD-matrix) with a removable cover was used. The obtained digital images of the cells were processed using the ImageJ software (Wayne Rasband, National Institutes of Health, USA; http://imagej.nih.gov/ij) with a 24-bit RGB color model (8 bits per channel).

Results. The adequacy of the developed approach was confirmed by the analysis of the above-listed drugs. It has been shown that the results obtained have no statistically significant differences from the values determined by the spectrophotometric method.

Conclusion. The possibility of using multisensor digital colorimetry for pharmaceutical analysis has been shown. The developed methods for the identification of the active substances can serve as a good supplement to more expensive traditional methods.

Keywords: dydrogesterone; troxerutin; ademetionine; digital multisensor colorimetry; barcode

Abbreviations: RGB – red, green, blue; MBTH – 3-methylbenzothiazolinone hydrazone; PCA – Principal Component Analysis; PC1 – Principal Component 1.

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RESEARCH ARTICLE

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МУЛЬТИСЕНСОРНЫЙ ЦВЕТОМЕТРИЧЕСКИЙ АНАЛИЗ ПРЕПАРАТОВ ДИДРОГЕСТЕРОНА, ТРОКСЕРУТИНА И АДЕМЕТИОНИНА С ИСПОЛЬЗОВАНИЕМ ШТРИХ-КОДОВ

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Цель. Разработка универсального экспрессного и доступного способа определения дидрогестерона, троксерутина и адеметионина в лекарственных препаратах методом мультисенсорной цифровой цветометрии с использованием уникального двумерного кода. Разработанный подход может быть применен для быстрого выявления фальсификатов лекарственных средств на предварительном этапе анализа (до использования более дорогого специализированного оборудования).

Материалы и методы. Для реализации предложенного подхода использованы субстанции дидрогестерона («Эбботт Биолоджикалз Б.В.», Нидерланды), троксерутина (АО «Интерфарма», Прага, Чехия), адеметионина (ООО «Фармамед», Москва, Россия), капсулы троксерутина 300 мг (ООО «Пранафарм», Самара, Россия), лиофилизат для приготовления раствора для внутривенного и внутримышечного введения «Гептрал»® (адеметионин) 400 мг («Эбботт Лэбораториз», ГмБХ, Германия), таблетки «Дюфастон»® (дидрогестерон) 10 мг («Эбботт Хелскеа Продактс Б.В.», Нидерланды). Метод мультисенсорной цветометрии реализован с использованием следующего набора из 8 сенсоров (C_1–C_8): интактный раствор – 96% (v/v) водный раствор этанола – C_1; 1 мМ спиртовой раствор антрахинонового зеленого (CAS#4403-90-1) – C_2; 0,2% водный раствор 3-метилбензотиазолинон-гидразона (CAS#1128-67-2) – C_3; 0,2% водный раствор метилоранжа (CAS#547-58-0) – C_4; 1 мМ спиртовой раствор сульфородамина В (CAS#3520-42-1) – C_5; 1 мМ спиртовой раствор 1-гидроксипирена (CAS#5315-79-7) – C_6; 1 мМ спиртовой раствор красного очаровательного АС (CAS# 25956-17-6) – C_7; 1 мМ водный раствор железа (III) хлорида – C_8. В качестве основы для чипа использовали прозрачные планшеты из полипропилена с плоским дном на 96 ячеек, объем ячейки – 350 мкл (Thermo Fischer Scientific, США, кат. № 430341). Для получения растровых изображений применяли офисный планшетный сканер Epson Perfection 1670 (CCD-матрица) со съемной крышкой. Полученные цифровые изображения ячеек обрабатывали в программе ImageJ (Wayne Rasband, National Institutes of Health, USA; http://imagej.nih.gov/ij) с использованием цветовой модели RGB 24 бит (8 бит на канал).

Результаты. Адекватность разработанного подхода подтверждена при анализе вышеперечисленных лекарственных препаратов. Показано, что полученные результаты не имеют статистически значимых отличий от величин, определённых спектрофотометрическим методом.

Заключение. Показана возможность использования мультисенсорной цифровой цветометрии для фармацевтического анализа. Разработанные способы определения действующих веществ могут служить хорошим дополнением к более дорогостоящим традиционным методам.

Ключевые слова: дидрогестерон; троксерутин; адеметионин; цифровая мультисенсорная цветометрия; штрих-код.

Список сокращений: RGB (КЗС) – красный, зеленый, синий; МБТГ – метилбензотиазолинон-гидразон; PC1 – главная компонента 1 / Principal Component 1; PCA – метод главных компонент / principal component analysis.

INTRODUCTION

For a preliminary rapid detection of counterfeits (even before using more expensive analytical equipment), it is advisable to practise simple, accessible and express methods. One of these methods is a digital colorimetry, based on the registration of electromagnetic radiation in the visible range of wavelengths by digital devices to get color raster images [1–6]. Digital colorimetry has become widespread in pharmaceutical analyses. In this area, the method is used to: analyze medicinal plants [7, 8]; assess the quality of collections, which include powders of medicinal plants [9–11]; determine the whiteness of powdered and tableted drugs [12]; identify the biologically active substances and drugs both by their own color and by the color of the products of color reactions used in pharmacopoeial tests [12]; identify the DOA and banned products [13, 14].

Digital colorimetry combines the availability of chemical test methods with a visual detection and a good performance of instrumental methods, primarily optical molecular spectroscopy. The extremely low cost of the analysis by this method is due to the possibility to measure the analytical signal using

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consumer digital optical devices not certified as measuring instruments [1, 5, 15, 16]. Despite the obvious advantages, the colorimetric method is not devoid of a number of limitations, the main one of which is its low selectivity [4, 17].

To increase the selectivity of the method, the use of molecular sensors was proposed [18]. It is advisable to use a cell of several chromogenic agents as sensors, in which a series of analytical reactions can be carried out simultaneously. The multisensor colorimetry method [19–29] is based on obtaining colored products of an analyte interaction with molecular sensors, getting information about their color characteristics, and then converting them into a discrete substance "barcode" that can be used for chemical analyses [17, 30].

A unique colorimetric two-dimensional code makes it possible to estimate both the nature and the content of active substances in the drugs at the minimum level of information noise [17, 30]. To form the "barcodes", it is advisable to choose such sensors and color channels, the values of the lightness of which correlate with the content of the analyte. Lightness shall mean the color coordinate on one of the color channels in the *RGB* system (varies in the range from 0 to 255).

The drugs of three different pharmacological groups were selected as the test objects. Dydrogesterone is synthetic progestogen that fully ensures the onset of the secretion phase in the endometrium in cases of endometriosis and dysmenorrhea. Troxerutin is a flavonoid, a phleboprotective drug that has venotonic, angioprotective, anti-inflammatory, anti-edema and antioxidant effects. Ademetionine is an antioxidant, hepatoprotective, detoxifying agent. The structural formulas of the active substances are shown in Fig. 1. The development of alternative methods for their identification, suitable for a preliminary screening analyses of drugs, is an important and urgent task of the pharmaceutical and analytical chemistry.

THE AIM of this study is to develop a universal method by multisensor digital colorimetric analysis of drugs of various pharmacological groups using dydrogesterone, troxerutin and ademetionine as examples. The developed complex of molecular sensors in combination with new approaches to the processing of analytical signals will make it possible to identify the above-mentioned active substances in the drugs.

MATERIALS AND METHODS

Study objects

To implement the proposed approach, the substances of dydrogesterone ("Abbott Biologicals B.V.", Netherlands), troxerutin (JSC "Interfarma", Prague, Czech Republic) and ademetionine (LLC "Farmamed", Moscow, Russia), troxerutin capsules 300 mg (LLC "Pranafarm", Samara, Russia), lyophilisate for an intravenous solution and an intramuscular administration of "Heptral"® (ademetionine) 400 mg ("Abbott Laboratories", GMBH, Germany), tablets "Duphaston"® (dydrogesterone) 10 mg ("Abbott Healthcare Products B.V.", Netherlands) were used.

Materials

For a quantitative analysis, a series of calibration solutions was prepared using the substances troxerutin and ademetionine (4.0-20.0 mg/ml) in increments of 4 mg/ml, dydrogesterone (1.0-3.0 mg/ml) in increments of 0.5 mg/ml. The concentration range had been selected in such a way that the content of the active substance in the real drug would be in the middle of the calibration curve.

The calibration solutions were analyzed by multisensor colorimetry using the following set of 8 sensors (C_1–C_8): an intact solution – a 96% (v/v) aqueous ethanol solution – C_1; 1 mM alcoholic solution of anthraquinone green (CAS#4403-90-1) – C_2; a 0.2% aqueous solution of 3-methylbenzothiazolinone hydrazone (MBTH) (CAS#1128-67-2) – C_3; a 0.2% methyl orange aqueous solution (CAS#547-58-0) – C_4; a 1 mM alcoholic solution of sulforhodamine B (CAS#3520-42-1) – C_5; a 1 mM alcoholic solution of 1-hydroxypyrene (CAS#5315-79-7) – C_6; 1 mM alcoholic solution of allura red AC (CAS#25956-17-6) – C_7; a 1 mM aqueous solution of of iron (III) chloride – C_8.

Equipment

Transparent flat-bottomed polypropylene plates with 96 cells [31–33], cell volume 350 µl (Thermo Fischer Scientific, USA, cat. No. 430341) were used as a base for the chip.

Using Biohit mLine dispensers (Sartorius, USA), 100 µL of alcohol solutions of substances, sensor solutions (C_1–C_8), and purified water were placed into the cells of the plate. The number of sensors was determined so that it would be possible to analyze the maximum number of samples on one plate (8 sensors by the number of rows of the plate).

For obtaining raster images, an Epson Perfection 1670 office flatbed scanner (CCD-matrix) with a removable cover was used. The plate with the samples was scanned using the Epson Scan software in the Professional mode (600 dpi resolution, 24-bit color depth). "Color restoration", "Unsharp mask filter" and "Descreening filter" options were disabled. To perform a digital colorimetric analysis using a 96-cell plate (Thermo Fischer Scientific, USA, cat. No. 1256604), a Teflon insert of 210×297×17 mm in size, was made with a center rectangular cut (128×86 mm) and was placed under the cover of an office A4 flatbed scanner. It made it possible to: expedite and formalize the procedure for placing the plate on the working glass table of the scanner; fix the coordinates and lighting conditions of the plate with an electroluminescent lamp built into the carriage; minimize the side stray illumination of the plate with substrates by external illumination sources; improve the accuracy of measuring results of plate raster images color channels lightness.

The difference in the color channels lightness between the analyte cell and the intact cell was used as an analytical signal. The obtained digital images of the cells were processed using the ImageJ software (Wayne Rasband, National Institutes of Health, USA; http://imagej.nih.gov/ij) using the RGB 24-bit color model (8 bits per channel), in each cell the central area was selected and 3 averaged values of lightness were obtained for it, one for each color channel of RGB. The choice of color channels was carried out empirically.

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RESULTS AND DISCUSSION

Semi-quantitative colorimetric analysis of troxerutin, dydrogesterone and ademetionine

The obtained values of the lightness of RGB-channels were processed in the MS Excel spreadsheet editor, the optimal threshold values of the difference in the lightness of the channels for the analyzed solution and the intact cell were chosen. The values above them were conventionally designated as "1" and below them as "0" (Table 1) and colorimetric "barcodes" were created (Table 2). When choosing the optimal threshold value for the difference in lightness, the following requirements were met: (1) the code must be unique; (2) the difference in coding between adjacent concentrations should be minimal (1-2 values). To meet these requirements, it is advisable to set individual thresholds for each channel. This problem was solved using MS Excel (Add-in "Search for a solution").

The presented one-dimensional "barcodes" can be clustered into a two-dimensional code (Table 3), which makes it possible both to estimate the nature and the content of the active substance in the drugs at the minimum level of the information noise. The interpretation of a two-dimensional code for the identification of the substances is possible both in visual and "instrumental" mode, for example, using a software "barcode" scanner on a smartphone after its preliminary setup. The latter mode is especially useful when processing large data sets to increase the reliability of the analysis results.

Thus, the technique of the semi-quantitative analysis of drugs can be reduced to comparing the code of the test solution with the corresponding code of a standard solution of the known concentration. Since the inaccuracy of the semi-quantitative analysis results is initially high, there is no need to use an inaccessible standard sample. It is just necessary to reproduce the described conditions of measuring the analytical signal and use a ready-made set of two-dimensional barcodes.

Colorimetric quantitative analysis of dydrogesterone, troxerutin and ademetionine drugs

For the quantitative analysis, it is advisable not to use all color channels and sensors, but only those the lightness values of which correlate with the analyte content. The coefficients of determination (r2) are calculated for all analytes, sensors and color channels, sensors and channels for which the value of r2 > 0.99 is a linearity criterion for the pharmaceutical analysis methods are identified. Thus, for the analysis of troxerutin, 4 color channels were selected (G4, G5, R7 and R8), for dydrogesterone – 5 channels (R2, G4, G6, B6 and R7), for ademetionine – 6 channels (R2, G2, R3, B3, G5 and G7).

To test the developed approach, a colorimetric analysis of the following drugs was carried out: tablets of dydrogesterone "Duphaston"® 10 mg, capsules of troxerutin 300 mg and lyophilizate of ademetionine "Heptral"® 400 mg. In order to select the optimal method for the identification of active substances, a comparison of the metrological characteristics of the methods using all the proposed color channels and sensors, was carried out. The content of the active substance in the drugs was determined by the calibration curve method. The results of the active substances identification in the indicated drugs using the developed approach, are presented in Table 4.

For all variants of colorimetric techniques, the equality of the means was proved using the modified Student's t-test for independent samples (P=0.95). The table shows that methods of the troxerutin identification using the R-channel of sensor 7, of ademetionine – the G- channel of sensor 2, and of dydrogesterone – the R-channel of sensor 2, have the best metrological characteristics. The presented data show that the results of the analysis of the drugs by method of multisensor digital colorimetry, accord well with the data declared by the manufacturer (obtained by high performance liquid chromatography and spectrophotometric method).

Using the technique of the principal component analysis for the assay of dydrogesterone, troxerutin and ademetionine drugs.

An approach in which the set of lightness values of color channels is considered as a kind of "colorimetric spectrum", seems promising. In this case the data can be processed using chemometric algorithms, of which the principal component analysis (PCA) is used most often. In this case, it is possible, on the one hand, to select all useful information from all sensors on all channels at once, on the other hand, the level of the information noise can be reduced and the accuracy of the analysis results can be increased.

To test chemometric approaches, a series of calibration solutions of the troxerutin and ademetionine substances (4.0–20.0 mg/ml) in increments of 4 mg/ml, and dydrogesterone (1.0–3.0 mg/ml) with a step of 0.5 mg/ml were used. The values of the first principal component (PC1) were calculated by the formulas.

For dydrogesterone:

$$PC1 = -0.01\cdot\Delta G_1 - 0.31\cdot\Delta R_2 - 0.02\cdot\Delta G_2 - 0.23\cdot\Delta B_2 - 0.01\cdot\Delta R_3 - 0.01\cdot\Delta G_3 - 0.35\cdot\Delta B_3 - 0.01\cdot\Delta R_4 - 0.21\cdot\Delta G_4 - 0.01\cdot\Delta B_4 + 0.01\cdot\Delta R_5 - 0.44\cdot\Delta G_5 - 0.40\cdot\Delta B_5 - 0.01\cdot\Delta R_6 - 0.09\cdot\Delta G_6 - 0.22\cdot\Delta B_6 - 0.24\cdot\Delta R_7 - 0.09\cdot\Delta G_7 - 0.02\cdot\Delta B_7 - 0.04\cdot\Delta R_8 - 0.46\cdot\Delta G_8 - 0.03\cdot\Delta B_8$$

For troxerutin:

$$PC1 = 0.02\cdot\Delta R_1 + 0.10\cdot\Delta G_1 + 0.05\cdot\Delta B_1 + 0.38\cdot\Delta R_2 + 0.01\cdot\Delta G_2 + 0.31\cdot\Delta B_2 + 0.17\cdot\Delta R_3 + 0.48\cdot\Delta G_3 + 0.21\cdot\Delta B_3 + 0.13\cdot\Delta G_4 + 0.14\cdot\Delta B_4 + 0.23\cdot\Delta R_5 + 0.16\cdot\Delta G_5 + 0.38\cdot\Delta B_5 + 0.17\cdot\Delta R_6 + 0.18\cdot\Delta G_6 + 0.23\cdot\Delta R_7 + 0.01\cdot\Delta G_7 + 0.02\cdot\Delta B_7 + 0.27\cdot\Delta R_8 - 0.02\cdot\Delta G_8 + 0.02\cdot\Delta B_8$$

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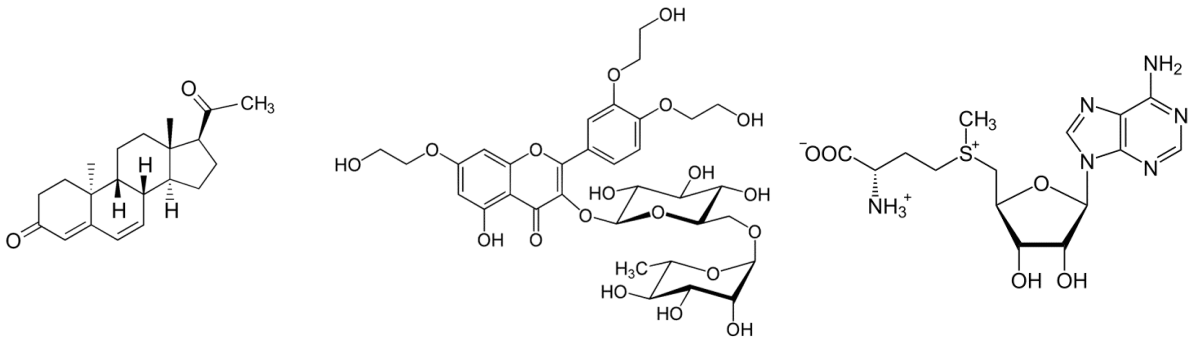

a) b) c)

Figure 1 – Structural formulas of dydrogesterone (a), troxerutin (b), ademetionine (c)

Table 1 – Colorimetric codes corresponding to various concentrations of dydrogesterone, troxerutin and ademetionine

Dydrogesterone					
c, mg/ml	ΔR_2	ΔG_4	ΔG_6	ΔB_6	ΔR_7
Threshold value of differences i n lightness	127	92	30	50	80
1.0	0	0	0	0	1
1.5	1	0	0	0	1
2.0	1	0	0	1	1
2.5	1	1	0	1	1
3.0	1	1	1	1	1
Troxerutin					
c, mg/ml	ΔG_4	ΔG_5	ΔR_7	ΔR_8	
Threshold value of differences in lightness	125	91	82	92	
4 or less	0	0	0	0	
8	1	0	0	0	
12	1	1	0	0	
16	1	1	1	0	
20	1	1	1	1	
Ademetionine					
c, mg/ml	ΔR_2	ΔG_2	ΔR_3	ΔB_3	ΔG_5	ΔG_7
Threshold value of differences in lightness	127	92	30	50	80	101
4	0	0	0	0	1	0
8	0	1	1	0	1	0
12	0	1	1	1	1	0
16	0	1	1	1	1	1
20	1	1	1	1	1	1

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Table 2 – Scale of "barcodes" corresponding to various concentrations of dydrogesterone, troxerutin and ademetionine

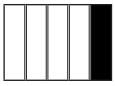
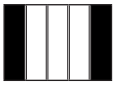
Dydrogesterone		Troxerutin		Ademetionine	
c, mg/ml	"Barcode"	*c*, mg/ml	"Barcode"	*c*, mg/ml	"Barcode"
1.0		4		4	
1.5		8		8	
2.0		12		12	
2.5		16		16	
3.0		20		20	

Table 3 – Two-dimensional "barcodes" for simultaneous analysis dihydrosterone, troxerutin and ademetionine

Active substance, c, mg/ml	C_1			C_2			C_3			C_4			C_5			C_6			C_7			C_8		
	R	G	B	R	G	B	R	G	B	R	G	B	R	G	B	R	G	B	R	G	B	R	G	B
Dydrogesterone (2.5 mg/ml)				■		■			■		■			■	■			■	■	■				
1.0																			■					
1.5				■															■					
2.0				■														■	■					
2.5				■						■								■	■					
3.0				■						■							■	■	■					
Troxerutin (16 mg/ml)		■	■	■		■		■		■	■	■	■	■					■	■	■			
4																								
8										■														
12										■			■											
16										■			■						■					
20										■			■						■			■		
Ademetionine (8 mg/ml)					■	■		■		■			■										■	■
4													■							■				
8					■								■							■				
12					■			■					■							■				
16					■			■					■							■				
20				■	■			■					■							■				

Note: C_1–C_8 – sensors; the dark fill of the cell corresponds to the presence of a signal, the light one – to its absence

Table 4 – Results of active substances identification in medicinal products by multisensor digital colorimetry using various color channels and sensors

Sensor and color channel	Active ingredient content, mg/unit		S_r (for digital colorimetry)
	Spectrophotometry (n = 3, P = 0.95)	Digital colorimetry (n = 11, P = 0.95)	
Dydrogesterone			
R_2	10.2 ± 0.1	11.1 ± 1.2	0.048
G_4		8.4 ± 1.0	0.053
G_6		7.0 ± 0.8	0.050
B_6		6.2 ± 0.6	0.042
R_7		14.4 ± 1.7	0.053
Troxerutin			
G_4	287 ± 2	294 ± 23	0.036
G_5		284 ± 25	0.040
R_7		290 ± 20	0.036
R_8		291 ± 18	0.028
Ademetionine			
R_2	391 ± 4	393 ± 42	0.048
G_2		395 ± 19	0.022
R_3		389 ± 27	0.031
B_3		388 ± 28	0.033
G_5		400 ± 35	0.040
G_7		376 ± 24	0.029

Table 5 – Results of multisensor colorimetric identification of active substances in drugs by the principal component analysis

Active ingredient content, mg/unit		S_r (for digital colorimetry)
Spectrophotometry (n = 3, P = 0.95)	Digital colorimetry (n = 11, P = 0.95)	
Dydrogesterone		
10.2 ± 0.1	11.0 ± 0.8	0.031
Troxerutin		
287 ± 2	290 ± 7	0.016
Ademetionine		
391 ± 4	388 ± 9	0.020

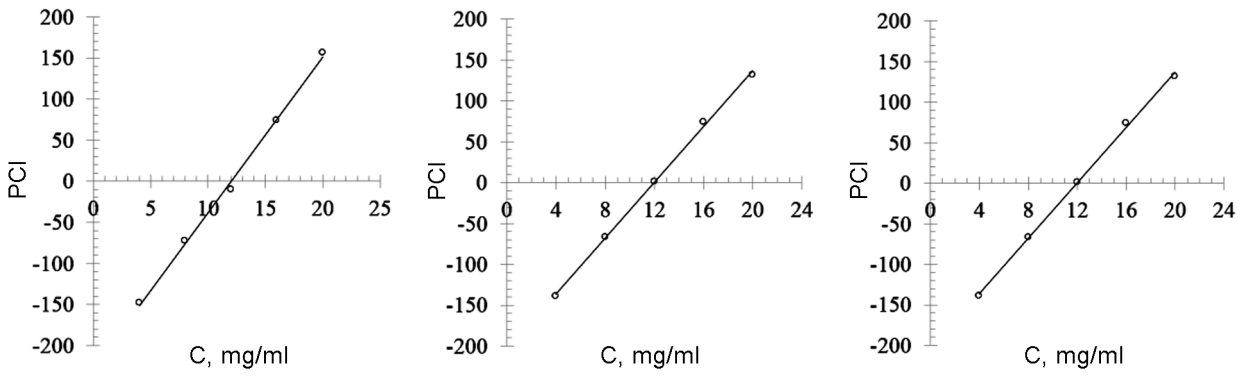


Figure 2 – Dependence of the first main component vs concentration of dydrogesterone (a), troxerutin (b), ademetionine (c) in calibration solutions

ОРИГИНАЛЬНАЯ СТАТЬЯ
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For ademetionine:

$$PC1 = 0.02 \cdot \Delta R_1 + 0.01 \cdot \Delta G_1 + 0.27 \cdot \Delta R_2 + 0.54 \cdot \Delta G_2 + 0.03 \cdot \Delta B_2 + \\ + 0.28 \cdot \Delta R_3 + 0.09 \cdot \Delta G_3 + 0.29 \cdot \Delta B_3 + 0.02 \cdot \Delta R_4 + 0.08 \cdot \Delta G_4 + 0.01 \cdot \Delta B_4 + \\ + 0.01 \cdot \Delta R_5 + 0.16 \cdot \Delta G_5 + 0.01 \cdot \Delta G_6 + 0.13 \cdot \Delta B_6 + \\ + 0.43 \cdot \Delta G_7 + 0.01 \cdot \Delta B_7 + 0.02 \cdot \Delta R_8 + 0.12 \cdot \Delta G_8 + 0.46 \cdot \Delta B_8$$

It can be notified that there is a linear correlation between the value of the first main component (PC1) and the content of dydrogesterone, troxerutin and ademetionine in calibration solutions (Fig. 2), which can be used to determine the content of these active substances in the drugs. The results of the analyses of the drugs for the identification of the active substances in the drugs using the developed approach, are presented in Table 5. The results obtained, accord well with the data declared by the manufacturer. Tables 4 and 5 show that the use of the principal component analysis improves the reproducibility of the analysis results in comparison with the use of the calibration dependence for the selected sensor and color channel.

CONCLUSION

An efficient approach (potentially having a wide application) has been proposed for a screening analysis of drugs of various pharmacological groups by multisensor digital colorimetry after a preliminary sample preparation. The simultaneous use of several chemical sensors in a chip provides sufficient selectivity. Discretization of the multisensor signal makes it possible to generate a unique barcode suitable for the identification of the active substances in drugs. The developed methods for the identification of active substances can serve as a good supplement to more expensive traditional methods.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTION

All authors have equally contributed to the research work.

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STUDY OF THE SAFETY OF ANTIANEMIC PREPARATIONS BY METHOD OF THE SYSTEM OF PROBLEMS RELATED TO MEDICINAL PREPARATIONS

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Iron deficiency is the most common micronutrient deficiency worldwide. Prevention and treatment of iron deficiency conditions are some of the most important health problems in many countries of the world. At the same time, the main problems for it remain the timely diagnosis, elimination of the cause, as well as the choice of replacement therapy with iron-containing drugs and correction of adverse reactions (ADR) that occur during their use.

The aim. This research aims to study the peculiarities of the development of antianaemic drugs ADRs in patients living in the territory of the Republic of Crimea.

Materials and methods. The objects of research were cases of ADR occurrence associated with the use of a group of antianaemic drugs and revealed during the 2009-2018 period in the territory of the Republic of Crimea. The main tasks in the analysis of notification forms were the study of the ADR severity, the causality assessment for suspected drugs and ADRs, as well as analysis of particular problems associated with the use of antianaemic drugs (Drug-related problems, DRP).

Results. Iron supplements in combination with other drugs became the leaders in the incidence of ADR among antianaemic drugs (28 cases, 42.4% of all cases of ADR). The largest number of cases was registered in patients aged from 18 to 30 years, with female patients prevailing. Among the clinical manifestations of ADR, the most cases were drug hypersensitivity reactions of varying severity (40 cases) and disorders of the gastrointestinal tract (18 cases). The study of the problems associated with the use of antianaemic drugs made it possible to determine that the highest rates of DRP values were observed with the use of iron preparations for parenteral use and cyanocobalamine. The minimal DRP values were observed when prescribing iron protein succinylate preparations.

Conclusion. The basis of pharmacotherapy for various types of anemias is the replenishment of iron and vitamin B_{12} (cyanocobalamin) depots. The effectiveness of the treatment in these cases largely depends on the patient's adherence to treatment, which is, in turn, depends on the frequency and severity of ADRs that occur during the use of antianaemic drugs.

Keywords: antianaemic drugs; iron supplements; adverse reactions; drug problems; DRP; dyspepsia

Abbreviations: DRP – Drug-related problems; WHO – World Health Organization; CI – confidence interval; MP – medicinal products; INN – international non-proprietary name; ADR – adverse drug reactions; MSS – musculoskeletal system; CR – causal relationship; DHR – drug hypersensitivity reactions, CNS – central nervous system

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ИЗУЧЕНИЕ БЕЗОПАСНОСТИ ПРИМЕНЕНИЯ АНТИАНЕМИЧЕСКИХ ПРЕПАРАТОВ С ИСПОЛЬЗОВАНИЕМ МЕТОДА СИСТЕМЫ ПРОБЛЕМ, СВЯЗАННЫХ С ЛЕКАРСТВЕННЫМИ ПРЕПАРАТАМИ

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Недостаток железа является наиболее распространенным дефицитом питательных микроэлементов во всем мире. Профилактика и лечение железодефицитных состояний является одной из важнейших проблем здравоохранения многих стран мира. Основными проблемами при этом остается своевременная постановка диагноза, устранение первопричины заболевания, а также выбор заместительной терапии препаратами железа и коррекции нежелательных реакций, возникающих при их применении.

Цель. Изучение особенностей развития нежелательных реакций антианемических препаратов у пациентов, проживающих на территории Республики Крым.

Материалы и методы. Объектами исследования являлись зарегистрированные за период 2009–2018 гг. на территории Республики Крым случаи возникновения нежелательных реакций при применении группы антианемических препаратов. Основными направлениями анализа карт-извещений стало изучение серьезности нежелательных реакций, достоверности причинно-следственной связи между подозреваемыми лекарственными препаратами и возникающими нежелательных реакций, а также проблем, связанных с применением антианемических лекарственных средств (Drug related problems, DRP).

Результаты. Лидерами по частоте развития нежелательных реакций среди антианемических препаратов стали препараты железа в комбинации с другими препаратами (28 случаев, 42,4% от всех случаев развития НР). Наибольшее количество случаев было зарегистрировано у пациентов в возрасте от 18 до 30 лет, при этом преобладали пациенты женского пола. Среди клинических проявлений нежелательных реакций преобладали случаи развития реакций лекарственной гиперчувствительности различной степени тяжести (40 случаев) и нарушения со стороны желудочно-кишечного тракта (18 случаев). Изучение проблем, связанных с применением антианемических препаратов, позволили определить, что наиболее высокие показатели значений DRP (DRP=10) наблюдались при применении препаратов железа для парентерального применения и цианокобаламина. Минимальные показатели значений DRP (DRP=6) наблюдались при назначении препаратов железа протеина сукцинилата.

Заключение. Основой фармакотерапии различных видов анемий является восполнение запасов железа и витамина B_{12} (цианокобаламина). Эффективность лечения пациентов при этом во многом зависит от их приверженности к лечению, которая обусловлена частотой и тяжестью нежелательных реакций, возникающих на фоне применения антианемических препаратов.

Ключевые слова: антианемические препараты; препараты железа; нежелательные реакции; проблемы, связанные с лекарственными препаратами; DRP; диспепсия

Список сокращений: DRP – проблемы, связанные с лекарственными препаратами; ВОЗ – Всемирная организация здравоохранения; ДИ – доверительный интервал; ЛС – лекарственные средства; МНН – международное непатентованное название; НР – нежелательные реакции; ОДА – опорно-двигательный аппарат; ПСС – причинно-следственная связь; РЛГ – реакции лекарственной гиперчувствительности; ЦНС – центральная нервная система.

INTRODUCTION

According to the World Health Organization (WHO) data, about 25% of the population worldwide is affected by anemia [1]. Therefore, iron deficiency is one of the most common health problems in the majority of the countries all over the world [2]. The main categories of patients with anemia are preschool children (47.4%; 95% confidence interval (CI) 45.7–49.1), pregnant women (41.8%; 95% CI 39.9–43,8), as well as non-pregnant women of childbearing age (30.2%; CI 28.7–31.6) [2]. Premenopausal women and patients with inflammatory bowel diseases are also high-risk iron loss and anemia groups [3].

The basis of pharmacotherapy for the conditions caused by iron deficiency, is oral and parenteral preparations of ferrous and ferric iron [4]. A high efficiency of these drugs, unfortunately, does not exclude the risk of developing adverse reactions (ADRs) during their use [5]. The oral administration of iron preparations is associated

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with the development of gastrointestinal disorders in patients [6]. This is confirmed by a meta-analysis conducted by Tolkien Z. et al. [3]. In the study, the incidence of constipation in the patients taking oral iron preparations, was 12%; diarrhea was observed in 8%, and nausea – in 11% of patients. This study also revealed that the frequency of gastrointestinal ADRs after peroral use of iron products was twice higher than in the patients of the control group, and 3 times higher than in the patients receiving iron preparations in the parenteral forms [3].

The administration of parenteral forms of iron preparations is much less frequently associated with gastrointestinal tract disorders. However, the risks of severe pseudoallergic reactions (the incidence of 1/200000 cases), manifested by severe hypotension, loss of consciousness, urticaria, and bronchospasm, are not excluded [7].

THE AIM is to study the development peculiarities of the adverse reactions (ARs) of antianemic drugs in the patients living in the territory of the Republic of Crimea.

MATERIALS AND METHODS

The objects of the study were ADRs cases registered for the period 2009–2018 in the territory of the Republic of Crimea, associated with the use of antianemia drugs. The information about ADRs for the chosen group of medicinal preparations (MPs) was extracted from the Autonomic Database of Adverse Reactions in Crimea (ARCADe – Adverse Reactions in Crimea, Autonomic Database). These were spontaneous reports. Database has a limited access.

The identification of adverse reactions to antianemia drugs was carried out using the codes of the Anatomical Therapeutic Chemical (ATC) classification of WHO[1] (ATC/DDD, Index 2019 (available at: https://www.whocc.no/atc_ddd_index/), Summary of Product Characteristics (SmPC) data from the State Registers of Medicines of the Russian Federation and Ukraine (for the cases registered before the accession of the Republic of Crimea to the Russian Federation). In accordance with the ATC classification, antianemia drugs have been assigned the B03 code. The studied pharmacological group includes the groups of iron preparations (B03A), vitamins B12 and folic acid (B03B), and other antianemia drugs (B03X).

The analysis of the ADRs severity was carried out in accordance with Article 4 of Federal Law No. 61-FZ dated April 12, 2010 «On the Circulation of Medicines»[2].

The study of the causal relationship (CR) reliability was carried out in accordance with the algorithms of Naranjo and Karch F.E., Lasagna L.[3] [8]. In accordance with the Naranjo algorithm, a degree of CR is assessed by a certain number of points received when answering a questionnaire. The degree of reliability, expressed in points, is classified as follows: certain (9 or more points), probable (5–8 points), possible (1–4 points), doubtful (0 or fewer points). Evaluation of CR using the Karch-Lasagna algorithm assumes an answer to 5 questions with their scoring and the selection of 5 main CR categories: certain (8 or more points), probable (6–7 points), possible (4–5 points), conditional (1–3 points), unlikely (0 or fewer points).

The type of adverse reactions was determined using two main classifications: the WHO (Rawlins-Thomson) classification and the Will-Brown one [9]. In accordance with the WHO classification, 6 main types of ADRs are distinguished (type A – dose-dependent ADRs, type B – dose-independent ADRs, type C – ADRs associated with a prolonged use of drugs, type D – delayed reactions, type E – withdrawal reactions, type F – 'no effect' reactions). The Williams and Brown classification includes 9 types of ADRs: type A – augmented dose-dependent, passing when the drug is withdrawn or its dose is reduced; type B – the action on microorganisms (for example, antibiotics administration causes candidiasis); type C – chemical/chronic ones, concentration-dependent; type D – ADRs associated with the delivery method or a drug form (for example, the occlusion of blood vessels by drug particles), type E – withdrawal reactions, physical dependence; type F – familial, reactions caused by a congenital metabolic defect; type G – a genetic damage; type H – hypersensitivity reactions; and type U – unclassified. The classification of ADRs proposed by the WHO is based on the predictability of ADRs and dose-dependence. The use of the Williams and Brown classification allows to more fully characterize ADRs, including the identification of the ones caused by a genetic damage, congenital metabolic defects, and the effect of a drug on microorganisms.

The second stage of the analysis of ADRs notification cards for antianemia drugs, was the study of problems associated with the use of drugs (Drug-related problems, DRPs). According to the Pharmaceutical Care Network of Europe (PCNE), a DRP is defined as "an event or circumstance associated with drug therapy that actually or potentially prevents the patient from achieving the desired results of pharmacotherapy"[4] [10–14]. The DRPs analysis was carried out using the updated version of the DRPs qualification system PCNE V9.0 [13], which allows assessing the problems, causes and interventions associated with DRPs [19]. The benefit of the ninth version of the PCNE V 9.0 system is the inclusion of category A (Acceptance) – "Interventions acceptance", in the analysis. The options for accepting interventions (code «A») are as follows: the intervention is acceptable, the intervention is not acceptable, or there is no information about the adoption of certain interventions.

[1] ATC/DDD Index 2019. Available at: https://www.whocc.no/atc_ddd_index/

[2] Federal Law of the Russian Federation No.61-FZ dated April 12 for 2010 "On the Circulation of Medicines". Electronic resource: http://www.consultant.ru/document/cons_doc_LAW_99350/ (link active on 10.12.2019).

[3] Standard operating procedure for monitoring the effectiveness and safety of drugs in medical organizations of the state healthcare system of the Moscow for general practitioners and nurses. Guidelines. – Edited by M.V. Zhuravleva. – Moscow. – 2019. 42 p.

[4] PCNE Classification for Drug-Related Problems V9. Available from: https://www.pcne.org/upload/files/417_PCNE_classification_V9-1_final.pdf)

The evaluation of the obtained DRPs results makes it possible to identify the main factors contributing to the development of complications after the use of drugs. Among such factors, an irrational choice of drugs, a violation of the dosage regimen, and the lack of taking into account the possible drug-drug interaction, can be singled out. The ADRs cases characterized by low DRPs indices, indicate a relative safety of pharmacotherapy for the patient. To determine the boundaries of the confidence intervals, the Clopper-Pearson method was used [15].

Each case of ADRs associated with the use of antianemia drugs was assessed by three researchers (Matveev A.V., Egorova E.A., Bekirova E.Yu.), in case of disagreement between them, the opinion of the fourth expert (Koniaeva E.I.) was taken into account.

RESULTS

To analyze the adverse reactions associated with the use of antianemia drugs (ATC code B03), 66 reports (2009–2018) were selected from the ARCADe regional database, which amounted to 0.96% of the total number of ADR cases registered for the corresponding period in the Republic of Crimea (6843 notification cards). The distribution of antianemia drugs by pharmacological groups was as follows: 53 cases (80.3%) of ADRs to iron preparations and 13 cases (19.7%) to vitamin B12 and folic acid preparations (Table 1).

It is important to note a high incidence of ADRs to iron supplements in combination with other drugs (28 cases, 42.4% of all cases of ADRs to antianemia drugs). Among sporadic representatives of combined drugs, the leader in the incidence of ADRs was a combination of iron sulfate (II) and ascorbic acid – 20 cases, less often ADRs were caused by the use of a combination of iron gluconate dihydrate and manganese and copper gluconate – 5 cases. Two cases were associated with the use of a combination of iron fumarate, cyanocobalamin, folic acid, pyridoxine hydrochloride, and sodium docusate; and 1 case was associated with the use of a complex of iron ammonium citrate with folic acid and cyanocobalamin.

The study of the gender characteristics of the ADRs developed after antianemia drugs, made it possible to determine that the majority of ADRs cases were observed in female patients (50 cases, 83.3%), which may be associated with a higher incidence of iron deficiency anemia caused by menstrual blood loss, childbirth, and lactation [16].

The analysis of the age categories of patients with registered antianemia drugs ADRs, was of scientific interest too. In 4 cases (4.5% of the total number of cases), ADRs were observed in pediatric patients (from 0 to 18 years old). In the remaining 62 notification cards, ADRs were observed in patients over 18 years of age. The distribution of the ADRs incidence rate in this age group is shown in Fig. 1.

The analysis of administration ways of antianemia drugs in patients with clinical manifestations of ADRs revealed the prevalence of the oral administration (43 cases, 65.2%). Much less often drugs were administered parenterally (intravenously – 4 cases, 6%; intramuscularly – 15 cases, 22.7%). In 4 cases, there was no information on the administration way of the suspected drugs.

Among the clinical manifestations of ADRs that occur in patients against the background of antianemia drugs, the cases of development of drug hypersensitivity reactions (DHSRs) of varying severity prevailed: urticaria, skin hyperemia – 36 cases (54.5%); angioedema – 2 cases (3.0 %); and anaphylactic shock – 2 cases (3.0%). In 18 cases (27.3%), patients had various clinical gastrointestinal tract symptoms (bloating, diarrhea, nausea, spastic pain). Hemodynamic disorders (weakness, hypotension) and disorders of the central nervous system (headache, dizziness) were observed much less frequently (3 cases of ADRs). The distribution of the remaining cases of ADRs according to their clinical manifestations is shown in Fig. 2.

The obtained data confirm the results of other studies on the possibility of DHSRs after a parenteral use of iron [17–20], which requires careful monitoring of the patient's condition during the infusion, and timely recognition and immediate medical intervention in case of acute hypersensitivity reactions.

A high incidence of DHSRs necessitated an additional analysis of cases based on concomitant allergic anamnesis of patients (household, contact or drug allergies). The number of patients with an allergic anamnesis was 2, in the remaining patients (64 cases) the allergic anamnesis was not complicated.

Another important factor contributing to the development of ADRs, is a simultaneous prescription of 2 or more drugs. According to Thong B. et al., the number of prescribed drugs is one of the most significant risk factors for ADRs development [21]. The results of the analysis of the notification cards made it possible to reveal that in most cases (32 cases, 48.5%) antianemia drugs were used as monotherapy, in 14 cases (21.2%) 1 concomitant drug was included in the patient's therapy list. Less frequently, a simultaneous prescription of 3 or more drugs was observed: 2 concomitant drugs – 5 cases (7.6%), 3 concomitant drugs – 8 cases (12.1%), 4 concomitant drugs – 4 cases (6.1%), 5 concomitant drugs – 2 cases (3%), and 6 concomitant drugs – 1 case (1.5%).

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Table 1 – Distribution of ADR cases by representatives of antianemia drugs

Drug	ATC-code	Amount of records, abs.	Amount of records, % of total antianemia drugs
Iron (II) products for peroral use			
Ferrous fumarate	B03AA02	5	7.6
Ferrous sulfate	B03AA07	2	3.0
Iron (III) products for peroral use			
Iron preparations for oral administration	B03AB	3	4.6
Iron (III) hydroxide polymaltose	B03AB05	2	3.0
Iron protein succinylate	B03AB09	2	3.0
Iron products for parenteral use			
Iron preparations for parenteral use	B03AC	11	16.7
Iron products in combinations with other products			
Iron products in combinations with other products	B03AE10	28	42.4
Cyanocobalamin and Folic acid			
Cyanocobalamin	B03BA01	12	18.2
Folic acid	B03BB01	1	1.5

Table 2 – Median, maximum and minimum DRPs values for antianemia drugs ADRs

Drug	Minimal DRP	Maximal DRP	Median DRP	Range
Iron (II) and iron (III) products for peroral use				
Ferrous fumarate	10	8	9	2
Iron preparations for per-oral use	9	9	9	0
Ferrous sulfate	9	10	9.5	1
Iron (III) hydroxide polymaltose	9	10	9.5	1
Iron protein succinylate	6	6	6	0
Iron products for parenteral use				
Iron products for parenteral use	6	12	10	6
Iron products in combinations with other products				
Iron products in combinations with other products	8	10	9	2
Cyanocobalamin and Folic acid				
Cyanocobalamin	9	14	10	5
Folic acid	10	10	10	0

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Table 3 – Total indices of the median, maximum and minimum values of DRPs for antianemia drugs ADRs in accordance with the standard qualification categories

Drug	Category "P"	Category "C"	Category "I"	Category "A"	Category "O"	Total DRP
Iron (II) and iron (III) products for peroral use						
Ferrous fumarate	1 (1:1)	1 (1:1)	5 (4:6)	1 (1:1)	1 (1:1)	9 (8:10)
Iron preparations for peroral use	1 (1:1)	1 (1:2)	5 (4:5)	1 (1:1)	1 (1:1)	9 (9:9)
Ferrous sulfate	1 (1:1)	1 (1:1)	5.5 (5:6)	1 (1:1)	1 (1:1)	9.5 (9:10)
Iron (III) hydroxide polymaltose	1 (1:1)	1 (1:1)	5.5 (5:6)	1 (1:1)	1 (1:1)	9.5 (9:10)
Iron protein succinylate	1 (1:1)	1 (1:1)	2 (2:2)	1 (1:1)	1(1:1)	6 (6:6)
Iron products for parenteral use						
Iron products for parenteral use	1 (1:1)	1 (0:3)	6 (2:7)	1 (1:1)	1(1:1)	10 (6:12)
Iron products in combinations with other products						
Iron products in combinations with other products	1 (1:1)	1 (1:1)	5 (4:6)	1 (1:1)	1 (1:1)	9 (8:10)
Cyanocobalamin and Folic acid						
Cyanocobalamin	1 (1:2)	1 (1:4)	6 (5:7)	1 (1:1)	1 (1:1)	10 (9:14)
Folic acid	1 (1:1)	1 (1:1)	6 (6:6)	1 (1:1)	1 (1:1)	10 (10:10)

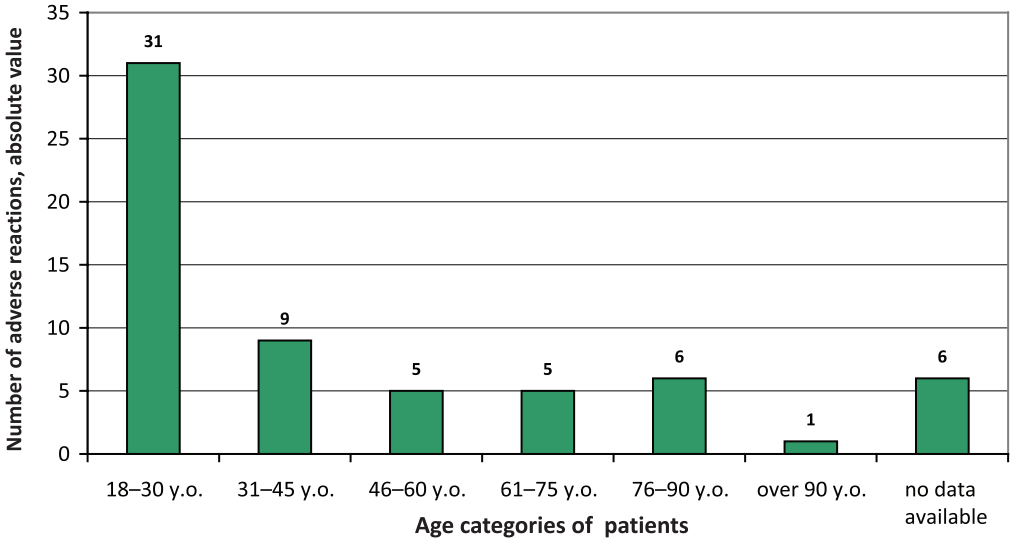

Figure 1 – Distribution of antianemia drugs ADRs in accordance with the age categories of patients

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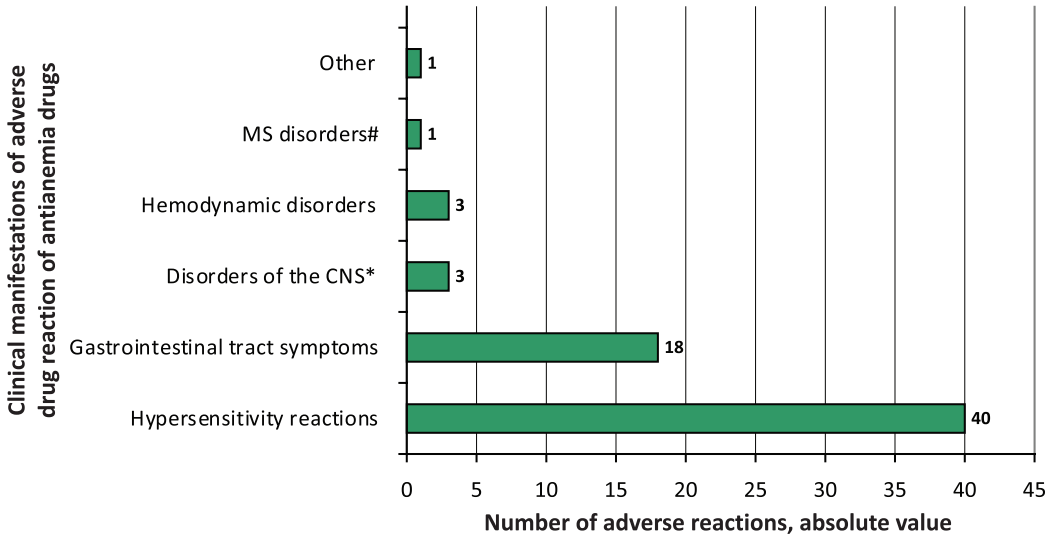

Figure 2 – Distribution of antianemia drugs ADRs in accordance with their clinical manifestations

Note: * – central nervous system, # – musculoskeletal disorders

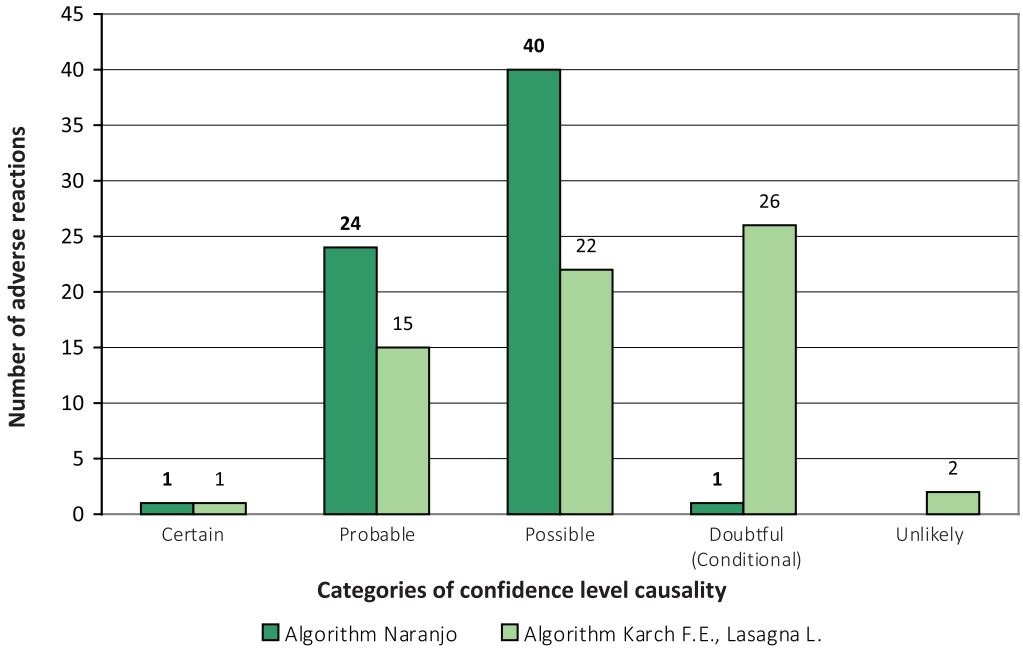

Figure 3 – Distribution of antianemia drugs ADRs in accordance with the degree of reliability of the causal relationship by the Naranjo and Karch F.E.-Lasagna L. algorithms

An important stage in assessing the safety of antianemia drugs is the identification and assessment of serious ADRs, which require a timely discontinuation of the drug therapy, hospitalization of the patient, and/or emergency pharmacotherapy. In accordance with the Federal Law of the Russian Federation No. 61-FZ dated April 12, 2010 «On the Circulation of Medicines», the category of serious adverse reactions includes such ADRs that lead to death, hospitalization, or prolongation of its terms, disability, or congenital anomalies, or a threat to the lives of patients[2].

The distribution of antianemia drugs ADRs in accordance with the criteria of their severity was represented by the following results: a threat to the patient's life – 4 cases (6.1%), temporary disability – 3 cases (4.5%), the need for hospitalization or prolongation of its terms – 4 cases (6.1%). Life-threatening conditions (2 cases of anaphylactic shock and 2 cases of angioedema) were caused by the administration of an iron (III) hydroxide sucrose complex (2 cases), iron (III) hydroxide polymaltose (1 case), and a combined preparation of iron (1 case for iron fumarate + cyanocobalamin + folic acid+ pyridoxine + sodium docusate). The need for hospitalization or its prolongation was due to the development of DHSRs in the form of a confluent rash, as well as severe dyspeptic ADRs (nausea and vomiting).

For the studied group of drugs, the incidence rate of serious ADRs was 8 cases (12.1%), which indicates a high degree of antianemia drugs safety. This is of particular importance in connection with the need for long-term anemia pharmacotherapy. The average duration of an oral iron supplementation course in iron deficiency anemia, ranges from 3 (mild IDA) to 6 months (severe IDA)[5] [22].

The study of the frequency and characteristics of pharmacological correction prescribed by doctors to stop antianemia ADRs made it possible to obtain the following results: the need for medical correction was necessary in 30 cases of ADRs (45.5%), in the remaining 36 cases (54.5%) additional drugs for the relief of ADRs clinical symptoms were not necessary. The main methods of the ADRs correction were antihistamines of the 1st and 2nd generations (Diphenhydramine, Chloropyramine hydrochloride, Loratadine), systemic glucocorticosteroids (Prednisolone, Dexamethasone, Hydrocortisone), and antiemetics.

The assessment of the drug safety also includes determining the degree of CR between the clinical and pharmacological characteristics of the drug and the clinical manifestations of ADRs[3] [8]. It is important to note that at the current moment a CR determination is the most important stage in the assessment of ADRs reports, which is carried out in national and regional pharmacovigilance systems in many countries of the world [23].

In this study, the causality assessment was carried out using the Naranjo and Karch F.E.-Lasagna L algorithms. The results of the CRs assessment of antianemia drugs and the undesirable consequences for patients, are presented in Fig. 3. The most frequent validation categories of CRs, according to the Naranjo algorithm, were probable and possible CRs. This factor indicates the presence of a validation relationship between the use of a drug and an ADR. The analysis of the notifications cards using the Karch F.E. and Lasagna L. algorithm, revealed a high frequency of possible and doubtful CRs, which could be explained by the absence of the time of an ADR occurrence as well as the lack of information on the results of re-challenge of the suspected drug.

The study of ADRs types was carried out using the WHO classification and the Williams and Brown classification. The analysis of ADRs types in accordance with the WHO classification made it possible to determine that in 42 cases (63.3%) there were dose-independent reactions (type B), in the remaining 24 cases there were dose-dependent ADRs (type A). The distribution of ADRs by type in accordance with the Williams and Brown classification made it possible to obtain similar results: hypersensitivity reactions (type H) were observed in 42 cases, and augmented, dose-dependent ones (type A) – in 24 cases.

[5] National standard of the Russian Federation. Patient management protocol. Iron-deficiency anemia. (Electronic resource). Available from: http://docs.cntd.ru/document/1200068753

In the study of the ADRs associated with the use of antianemia drugs, the next stage was the study of drug-related problems (DRPs) [29]. The calculation of the total DRPs values for the ADRs cases made it possible to obtain the following results: DRPs values within 5-8 points were observed in 11 cases (6 DRPs – 6 cases, 7 DRPs – 1 case, and 8 DRPs – 4 cases). In most of the notification cards the number of DRPs was in the range of 9-10 (25 cases, respectively). In 6 cases, the DRPs values were higher than 11 (11 DRPs – 2 cases, 12 DRPs – 2 cases, 13 DRPs – 1 case, and 14 DRPs – 1 case). These factors may indicate the likelihood of incorrect selection of doses when prescribing antianemia drugs or an irrational choice of the drug itself. The total number of DRPs for all cases of ADRs was 623 DRPs; therefore, the average number of DRPs per patient is 9.4 DRPs.

Further on, a quantitative analysis of the problems related to the use of various antianemia drugs was carried out according to the main categories of the ATC classification. For each group of antianemia drugs, the minimum and maximum values, as well as the median DRPs, were calculated (Table 2).

The study of sporadic categories of the DRPs system («P», «C», «I», «A», «O») revealed that for all the studied drugs, the maximum number of problems associated with drugs, was recorded in the section «I» (Interventions). High DRPs values in the presented cases may be due to the interventions on behalf of the doctor in the form of withdrawal or reduction of the dose of the suspected drug and the prescription of additional pharmacotherapy to correct the ADRs.

The analysis of the final DRPs values for each antianemia drug showed that the maximum DRP value was observed for parenteral iron products (10 DRPs), cyanocobalamin (10 DRPs), and folic acid (10 DRPs) (Table 3). The study of these cases confirmed the irrational prescription of cyanocobalamin in chronic pancreatitis (self-treatment) and the irrational prescription of iron for the lumbar dystopia of the right kidney, which caused such high rates. The minimum DRPs values (6 problems) were observed with the use of the same agents. Such ADRs were associated with allergic reactions in case of their rational prescription.

The results of the analysis presented in Table 3 show that the largest range between the minimum and maximum DRPs values was usual for parenteral iron products (max:min – 6:12), and the lowest for iron protein succinylate (max:min – 6:6), peroral iron drugs (9:9) and folic acid (10:10).

DISCUSSION

The study of safety criteria for antianemia drugs, including iron preparations, is an urgent problem all over the world. This is primarily due to the fact that iron deficiency anemia is a clinical symptom of many diseases, such as chronic renal failure, cancer, chronic heart failure, and inflammatory bowel disease [25].

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The research by Goodnough L.T. (2012) confirms the authors' results on a high rate incidence of gastrointestinal ADRs after the use of oral forms of iron products. According to the authors' data, the incidence of such disorders was more than 30% among the treated patients [26]. A parenteral administration of iron drugs, according to data of foreign researchers, is characterized by a less favorable safety profile. First of all, an intravenous administration of iron drugs may be associated with the risk of anaphylactic reactions [27; 28]. According to Szebeni J. et al. (2015), the prevalence of hypersensitivity reactions after a parenteral administration of iron products is about 0.1% [29]. A higher incidence of hypersensitivity reactions (1.4%) associated with the use of iron, was determined in a retrospective study conducted by Australian researchers. They had analyzed the medical records of patients in the municipal healthcare network in the period from January 1, 2010, to December 31, 2019 [30].

The researchers distinguish the following main risk factors for the development of hypersensitivity reactions after the administration of iron preparations: a rapid rate of iron infusion, an allergic anamnesis of the patient, severe atopy, and systemic inflammatory diseases [31]. Numerous studies have also confirmed the authors' data on a more frequent development of serious hypersensitivity reactions in female patients, which is due to the high prevalence of anemia and the need to prescribe iron supplements to this category of patients [32]. In this case, the study by Qassim A. et al. (2018) aimed at studying the efficacy and safety of an intravenous administration of iron polymaltosate preparations in the treatment of iron deficiency during pregnancy, should be mentioned. A retrospective cohort study of 213 pregnant women was conducted from January 2014 to January 2016 at a tertiary clinical hospital. The data on the ADRs development related to iron supplements, were collected from medical papers and electronic records. The results of the study confirmed a rather high rate incidence of ADRs after an intravenous administration of drugs (23.5%), the main of which were local reactions at the administration site (n = 8; 16%), headache (n = 8; 16%), symptomatic hypotension (n = 8; 16%), back pain (n = 7; 14%), and heartburn (n = 6; 12%). In one case, the administration of iron polymaltosate was associated with a severe anaphylactic reaction, manifested by wheezing, chest tightness, and an increased blood pressure. It should be noted that 32 women (15%) experienced side effects requiring discontinuation of treatment and therapy of ADRs symptoms [33].

Another adverse consequence of the use of parenteral iron-containing medicinal products is the ability to reduce chemotaxis as well as the ability of polymorphonuclear cells to the phagocytic activity, which leads to an increase in the risk of infectious processes [32]. The authors' retrospective study of spontaneous messages in the Republic of Crimea did not reveal such ADRs, which may be due to a rather low frequency rate, as well as the severity of their recognition at the post-authorization stage.

The results of the other studies confirm a low incidence rate of ADRs associated with the use of antianemia drugs. However, a parenteral administration of iron preparations can be accompanied by the development of severe anaphylactic reactions that threaten the life of patients, and requires a timely emergency aid to patients with such manifestations of ADRs.

CONCLUSION

The analysis of the ADRs notification cards, recorded in the Republic of Crimea, made it possible to reveal that the most frequent causes of ADRs development in the group of antianemia drugs, are combined iron preparations. The most common clinical manifestations of ADRs were drug hypersensitivity reactions (40 cases) and dyspeptic disorders (18 cases). The frequency rate of serious adverse reactions was 8 cases (12.1% of the total number of ADRs), which indicates a fairly high safety profile of antianemia drugs.

The study of the problems related to the use of antianemia drugs determined that the highest rates of DRPs values were observed during the parenteral use of iron preparations, use and cyanocobalamin. The minimum indicators of DRPs values were observed in the prescription of iron protein succinylate products.

The optimization of pharmacotherapy and prevention of DRPs makes it possible to reduce the incidence rate of ADRs, and significantly increase patient adherence to the treatment, which is essential for long-term maintenance therapy of iron deficiency and megaloblastic anemias.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

A.V. Matveev – work at the concept and design of the study, statistical processing of the results, translation; E.A. Egorova – statistical processing of the results, text writing; E.I. Konyaeva – text writing, editing; E.Yu. Bekirova – text writing, statistical processing of the results; L.A. Adzhimamutova – processing of the research results.

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RESEARCH ARTICLE

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MOLECULAR DESIGN OF N-ACYL DERIVATIVES OF 2-(2-OXOPYROLIDIN-1-YL)-ACETAMIDE WITH GABA-ERGIC AND GLUTAMATERGIC ACTIVITIES

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The first of the most successfully implemented nootropic drugs in medical practice is piracetam, which should be attributed to cyclic derivatives of gamma-aminobutyric acid. The production of new piracetam derivatives with high nootropic activity is a promising direction in the development of new neuroprotective drugs.

The aim of the study is to predict GABA-ergic and glutamatergic activities of N-acyl derivatives of 2-(2-oxopyrolidin-1-yl)-acetamide by a molecular docking method through the energy analysis of interaction of modeled structures with $GABA_A$ and AMPA receptors with their subsequent targeted synthesis.

Materials and methods. The objects of the research are new N-acyl derivatives of 2-oxo-1-pyrrolidineacetamide and a virtual model of the $GABA_A$ receptor of the *Homo sapiens* organism with the identification code 6D6U and a three-dimensional model of the AMPA-receptor of the *Rattus norvegicus* organism with the identification code 3LSF from the RCSB PDB database. The simulated compounds were designed in the HyperChem 8.0.8 program. This program was also used to optimize geometry using the force field of molecular mechanics MM+. Molecular docking was carried out using the Molegro Virtual Docker 6.0.1 program. The preparation of N-acyl derivatives of 2-(2-oxopyrrolidin-1-yl)-acetamide was carried out by the interaction of 2-(2-oxopyrrolidin-1-yl)-acetamide with an excess of the corresponding anhydride under conditions of acid catalysis.

Results. Based on the results of molecular docking, a high affinity of all simulated compounds for the binding site of $GABA_A$ and AMPA receptors can be estimated. According to the predict, the maximum GABA-ergic activity should be expected for (N-[2-(2-oxopyrrolidin-1-yl)-acetyl]-butyramide. N-acyl derivatives of 2-oxo-1-pyrrolidineacetamide form a more stable complex with amino acid residues Arg207, Phe200, Thr202, Tyr97, Tyr157, Tyr205 and Phe65 of the $GABA_A$ receptor binding site than the GABA molecule. In terms of the minimum interaction energy, the N-acyl derivatives of 2-(2-oxopyrrolidin-1-yl)-acetamide are superior to a number of known ligands such as GABA, piracetam, anipiracetam, picamilon and pramiracetam. The tested compounds have also shown a high affinity for the binding site of the AMPA receptor. The leader compound is also the compound PirBut, as in the case of the $GABA_A$ receptor.

Conclusion. Molecular modeling of the ligands interaction with the active binding site of gamma-aminobutyric acid of the $GABA_A$ receptor by molecular docking showed that all virtual N-acyl derivatives of 2-oxo-1-pyrrolidineacetamide can exceed a number of nootropic drugs by activity. In the course of molecular design, a method for predicting a glutamatergic activity for 2-pyrrolidone derivatives has been developed. It suggests a significant nootropic activity for N-[2-(2-oxopyrrolidin-1-yl)-acetamide amides.

Keywords: 2-(2-oxopyrolidin-1-yl)-acetamide; N-acyl derivatives; $GABA_A$ receptor; AMPA receptor; nootropics; Molecular design; molecular docking; structural pharmacology; QSAR

Abbreviations: GABA – gamma-aminobutyric acid; CNS – central nervous system; BAC – biologically active compound; BBB – blood-brain barrier; IC50 – The half maximal inhibitory concentration.

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МОЛЕКУЛЯРНОЕ КОНСТРУИРОВАНИЕ N-АЦИЛЬНЫХ ПРОИЗВОДНЫХ 2-(2-ОКСОПИРОЛИДИН-1-ИЛ)-АЦЕТАМИДА, ОБЛАДАЮЩИХ ГАМК-ЕРГИЧЕСКОЙ И ГЛУТАМАТЕРГИЧЕСКОЙ АКТИВНОСТЯМИ

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Первым из наиболее успешно внедренных в медицинскую практику ноотропных лекарственных средств является пирацетам, который следует отнести к циклическим производным γ-аминомасляной кислоты. Получение новых производных пирацетама обладающих высокой ноотропной активностью, представляет собой перспективное направление при создании новых нейропротекторных препаратов.

Цель. Прогноз ГАМК-ергической и глутаматергической активности N-ацилпроизводных 2-(2-оксопиролидин-1-ил)-ацетамида методом молекулярного докинга посредством анализа энергии взаимодействия моделируемых структур с $ГАМК_А$- и AMPA-рецепторами с последующим их целенаправленным синтезом.

Материалы и методы. Объектами исследования являются новые N-ацильные производные 2-оксо-1-пирролидинацетамида и виртуальная модель $ГАМК_А$-рецептора организма *Homo sapiens* с идентификационным кодом 6D6U и трехмерная модель AMPA-рецептора организма *Rattus norvegicus* с идентификационным кодом 3LSF из базы данных RCSB PDB. Моделируемые соединения построены в программе HyperChem 8.0.8. С помощью этой программы также была проведена оптимизация геометрии с использованием силового поля молекулярной механики MM+. Молекулярный докинг осуществлялся посредством программы Molegro Virtual Docker 6.0.1. Получение N-ацильных производных 2-(2-оксопирролидин-1-ил)-ацетамида осуществлялось взаимодействием 2-(2-оксопирролидин-1-ил)-ацетамида с избытком соответствующего ангидрида в условиях кислотного катализа.

Результаты. По результатам молекулярного докинга можно судить о высоком сродстве всех моделируемых соединений к сайту связывания $ГАМК_А$- и AMPA-рецепторов. Согласно прогнозу, максимальную ГАМК-ергическую активность следует ожидать у (N-[2-(2-оксопирролидин-1-ил)-ацетил]-бутирамида. N-ацильные производные 2-оксо-1-пирролидинацетамида образуют более устойчивый комплекс с аминокислотными остатками Arg207, Phe200, Thr202, Tyr97, Tyr157, Tyr205 и Phe65 сайта связывания ГАМК $ГАМК_А$-рецептора, чем молекула ГАМК. По величине минимальной энергии взаимодействия N-ацильные производные 2-(2-оксопирролидин-1-ил)-ацетамида превосходят целый ряд известных лигандов, таких, как ГАМК, пирацетам, анипирацетам, пикамилон и прамирацетам. Также исследуемые соединения показали высокое сродство к сайту связывания AMPA-рецептора. Соединением-лидером также является соединение PirBut, как и в случае с $ГАМК_А$-рецептором.

Заключение. Молекулярное моделирование взаимодействия лигандов с активным сайтом связывания гамма-аминомасляной кислоты $ГАМК_А$-рецептора методом молекулярного докинга показало, что все виртуальные N-ацильные производные 2-оксо-1-пирролидинацетамида по активности могут превышать целый ряд ноотропных лекарственных препаратов. В ходе молекулярного конструирования разработана методика прогнозирования глутаматергической активности для производных 2-пирролидона. Она позволяет предположить значительную ноотропную активность для амидов N-[2-(2-оксопирролидин-1-ил)-ацетамида.

Ключевые слова: 2-(2-оксопиролидин-1-ил)-ацетамид; N-ацильные производные; $ГАМК_А$-рецептор; AMPA-рецептор; ноотропы; молекулярное конструирование; молекулярный докинг; структурная фармакология, QSAR

Список сокращений: ГАМК – гамма-аминомасляная кислота; ЦНС – центральная нервная система; БАС – биологически активные соединения; ГЭБ – гематоэнцефалический барьер; IC50 – концентрация полумаксимального ингибирования

INTRODUCTION

In recent years, the requirement of a systematic search for efficient therapeutic substances targeting the human CNS diseases associated with emotional disorders has been recognized. Among the drugs currently used in clinical practice are those with neuroprotective effects on the brain function integrity, and beneficial for the resistance of neurons to aggressive endogenous and exogenous factors. Specifically, an interesting group of substances identified as “nootropics” have been

proposed to improve mental performance, memory, and other cognitive CNS processes. Nootropic drugs are shown to enhance the resistance of nerve cells to the effects of hypoxia, intoxication, and post-traumatic brain injury [1].

An important advantage of nootropic drugs is their low toxicity and compatibility with CNS drugs from other pharmacological groups that affect the central nervous system, and almost a complete absence of undesirable side effects [2].

Nootropics are involved in the enhancement of neuronal processes, the exchange of nucleic acids and proteins in cells, the adenosine triphosphate production, glucose transport across the blood-brain barrier (BBB) and its further utilization in brain tissues [3].

To search for new biologically active compounds (BACs) that affect the GABA-ergic and/or glutamatergic systems, it is advisable to combine various methods of molecular modeling. For example, the combination of molecular logical descriptors and a structure-based analysis of molecular databases comprising ligands with known activities enables identification of common structural fragments/motifs ("pharmacophores") responsible for a certain type of activity. The combination of conformational analysis and molecular docking methods makes it possible to identify the preferable ligand positions at protein binding sites and to find correlations with the strength of the pharmacological effect of interest.

THE AIM of the study is to predict a GABA-ergic and glutamatergic activities of N-acyl derivatives of 2-(2-oxopyrolidin-1-yl) -acetamide by a molecular docking method through the energy analysis of interaction of modeled structures with $GABA_A$ and AMPA receptors with their subsequent targeted synthesis.

MATERIALS AND METHODS

The geometry optimization was carried out by using the MM+ molecular mechanics method as implemented in the HyperChem 8.0.8 program [4]. The ligand-receptor interactions at the gamma-aminobutyric acid binding site of the $GABA_A$ receptor and at the active site of the AMPA receptor were calculated using MolDockScore algorithm implemented in the Molegro Virtual Docker 6.0.1 program [5]. In the molecular docking protocol used in this study, 300 most stable conformations of N-acyl derivatives of 2-(2-oxopyrolidine-1-yl)-acetamide were docked into the 12-nm binding area of the $GABA_A$ and the AMPA receptors. The initial 3D structures of the $GABA_A$ and the AMPA receptors were taken from the Protein Data Bank, with the PDB codes 6D6U [6] and 3LSF [7], respectively.

Molecular docking to the $GABA_A$ receptor-binding site

The biological target of docking is the $GABA_A$ receptor, which is one of the family of Cys-loop receptors containing disulfide bonds between two cysteine residues. All the described GABA receptors are polymorphic protein formations; its structure largely depends on their localization in the tissues of the body. According to the modern classification, GABA receptors are divided into two groups – ionoform receptors of the $GABA_A$/$GABA_C$ type and metabotropic $GABA_B$ receptors [8].

The supramolecular structure of the $GABA_A$ receptor is a heteropentameric glycoprotein complex. The structure of the ionotropic receptor can include 7 types of subunits: α, β, γ, δ, ε, π and θ. In turn, the α subunit is represented by 6 isoforms, β and γ include 3 isoforms each, and the other types of subunits in the $GABA_4$ receptor have 1 isoform each. In the mammalian brain, the $GABA_A$ receptor is a pentamer formed by two α-and β-subunits and one γ-subunit (Fig. 1) [9]. Each subunit of the ionotropic channel has a tertiary structure, which is represented by the order of 400 amino acid residues. The subunit includes an N-terminal extracellular domain and 4 transmembrane domains – M1, M2, M3 and M4, which have a structural organization in the form of α-helices. It is the N-terminal domain that has numerous sites for binding various ligands, which can be represented by gamma-aminobutyric acid, benzodiazepines, barbiturates, and neuronal hormones [10]. It is assumed that the transmembrane domains M2 and M3 are involved in ligand binding and ion channel modulation [11].

Molecular docking to the AMPA receptor binding site

Previously, researchers found that amino acids P494, S497, S754, S729, D760, Y424 and N764 are responsible for the process of positive allosteric modulation of the AMPA receptor. In this case, the piracetam molecule can occupy three pharmacologically active locations at the binding site of the AMPA receptor. These spatial arrangements of piracetam molecules are located in close proximity to each other. In the first case, the piracetam molecule forms bonds with amino acids P494, S497 and S754; in the second case, it mainly interacts with amino acids D760 and Y424, but also binds to amino acid S729. Herewith, the first and second locations of the piracetam molecule are mutually exclusive. In the third case, the piracetam molecule interacts with amino acids S729, D760 and N764 [7]. Thus, only two piracetam molecules can simultaneously occupy pharmacologically active locations at the binding site of the AMPA receptor. In the study, the second location of piracetam was selected at the binding site of the AMPA receptor, and amino acids S729, D760, Y424 and N764 were designated as Ser 217, Asp 248, Tyr 35 and Asn 252, respectively. Two possible simultaneous variants of the location of the piracetam molecule at the binding site of the AMPA receptor in the 3LSF protein-ligand complex are shown in Fig. 2.

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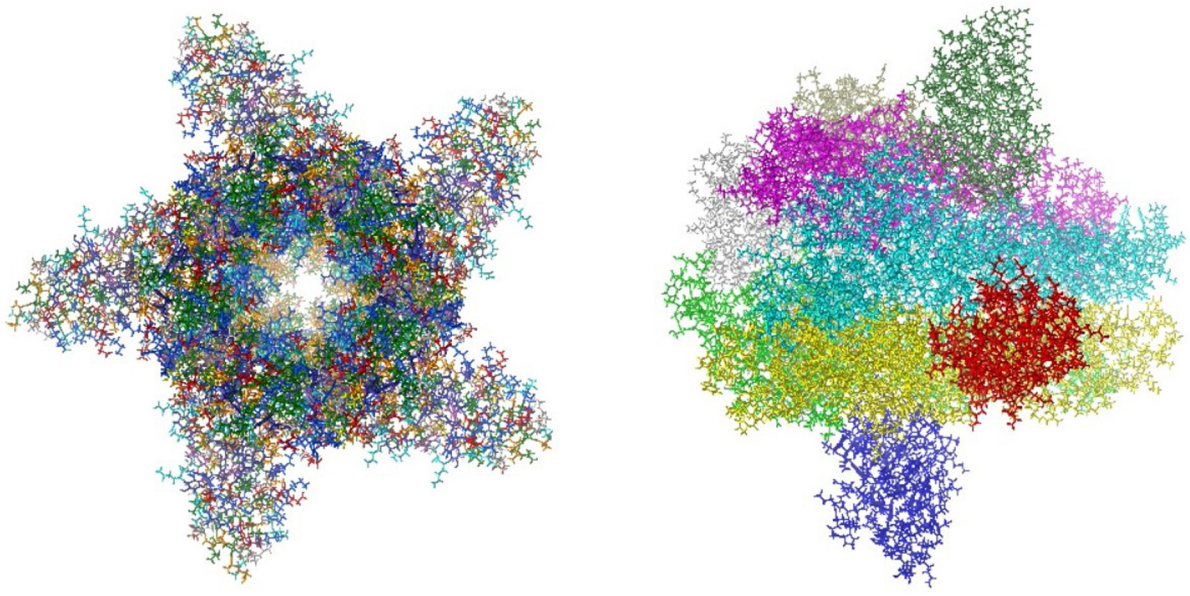
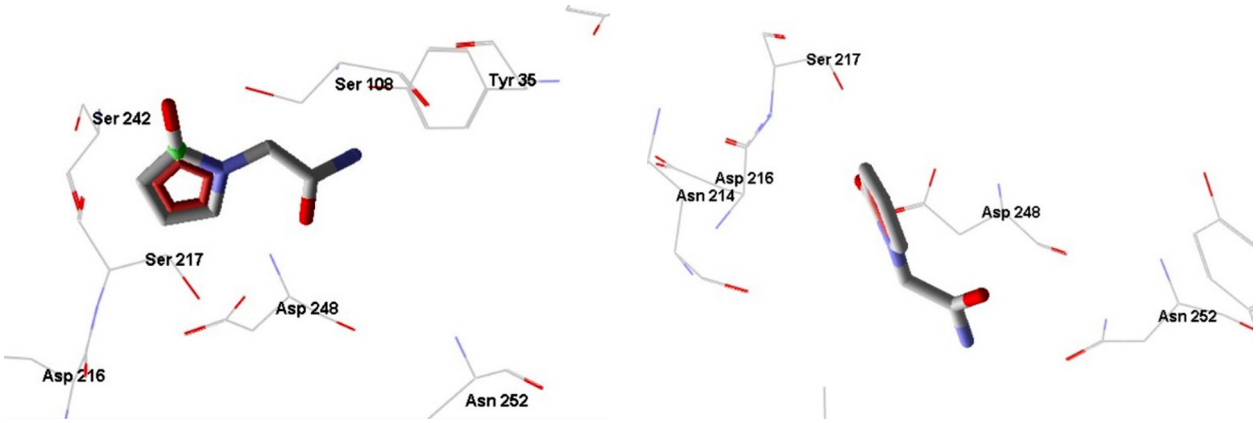


A B

Figure 1 – Molecular structure of the $GABA_A$ receptor

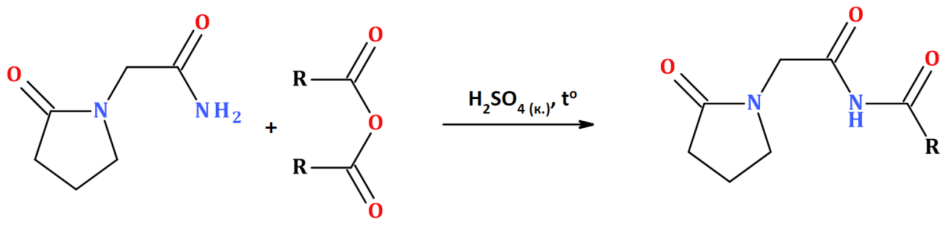
Note: A is a horizontal position of the $GABA_A$ receptor in the plane; B is a vertical one



A B

Figure 2 – Location of the piracetam molecule at the binding site of the AMPA receptor in the 3LSF protein-ligand complex

Note: A – the second location option. B – the third location option



R = CH_3 (PirAc); C_2H_5 (PirPr); C_3H_7 (PirBut)

Figure 3 – Synthesis of N-acyl derivatives of 2-oxo-1-pyrrolidinacetamide

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Table 1 – Structural formulas of ligands from the Binding DB with corresponding K_i indices

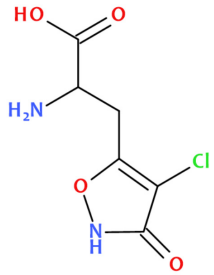
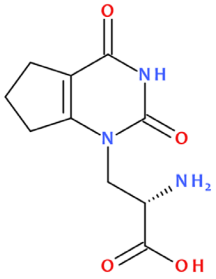
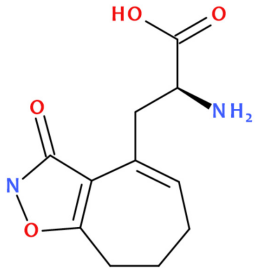
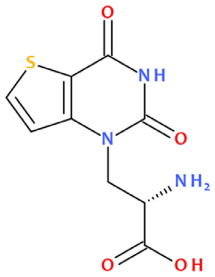
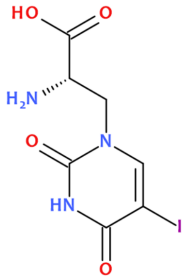
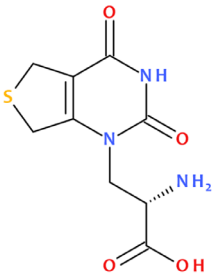
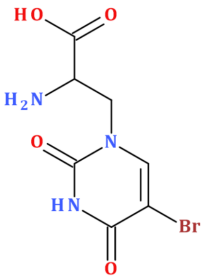
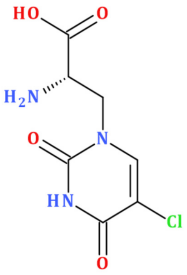
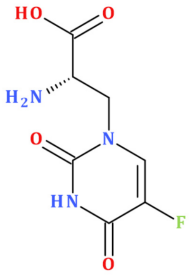
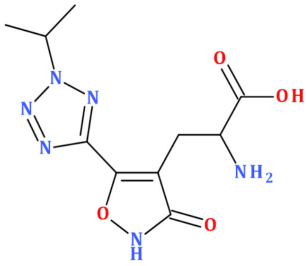
Ligand code, structural formula, K_i nM	Ligand code, structural formula, K_i nM	Ligand code, structural formula, K_i nM	Ligand code, structural formula, K_i nM
BDBM50128264, 370 	BDBM50107595, 218 	BDBM50126764, 175 	BDBM50252922, 112 
BDBM50060627, 105 	BDBM50252920, 80 	BDBM50252873, 61 	BDBM50060632, 45 
BDBM50060635, 4 	BDBM50166287, 2.9 		

Table 2 – The minimum value of the ligand-receptor interaction of the predicted ligands in the active center of the $GABA_A$ receptor

Ligand	Minimum energy of ligand-receptor complex formation, kcal/mol
GABA	–71.708
Piracetam	–86.509
PirAc	–88.691
PirPr	–95.354
PirBut	–95.507
Aniracetam	–81.509
Fonturacetam	–97.105
Phenibut	–94.692
Picamilonum	–78.629
Pramiracetam	–76.046

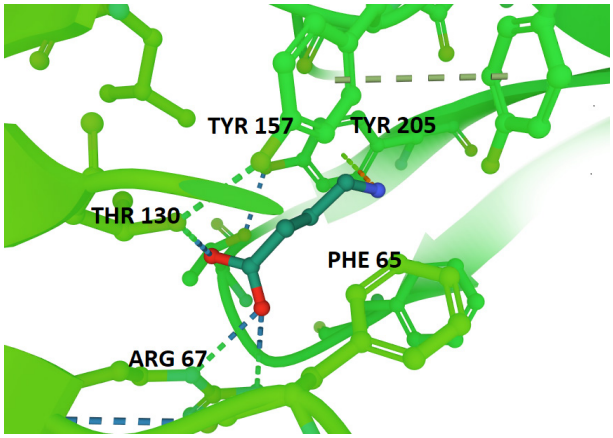


Figure 4 – Location of the gamma-aminobutyric acid molecule in the active center of the $GABA_A$ receptor

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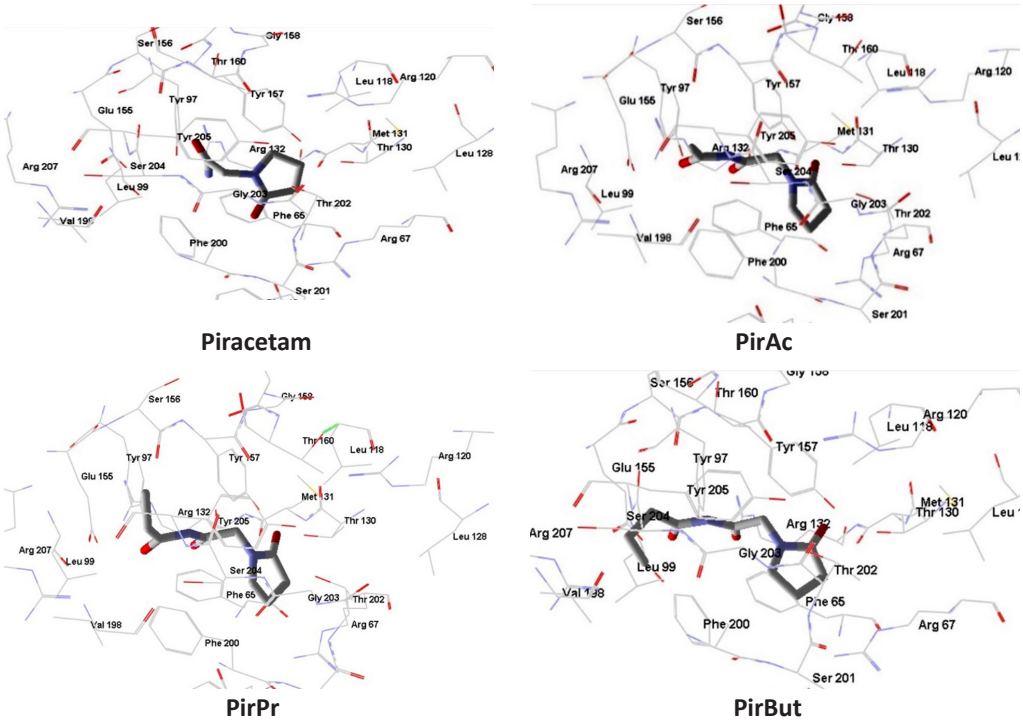

Figure 5 – Location of piracetam and its N-acyl derivatives in the active center of the $GABA_A$ receptor according to the results of molecular docking

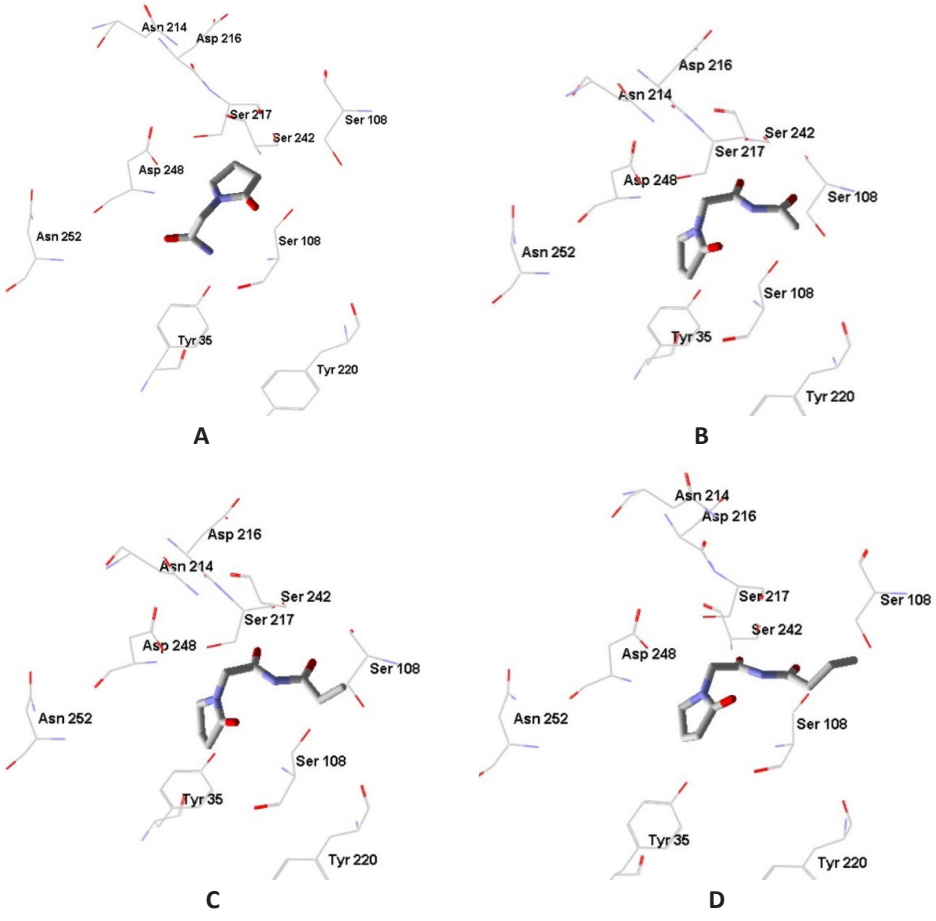

Figure 6 – The location of ligands at the binding site of the AMPA receptor according to the results of molecular docking

Note: A – piracetam; B-PirAc; C – PirPr; D – PirButt

Table 3 – The formation energy of ligand bonds with amino acids Thr 130, Arg 67, Tyr 205, Tyr 157 and Phe65, kcal/mol

Ligand	Amino acid		Sum	Amino acid			Sum
	Thr 130	Arg 67		Tyr 205	Tyr 157	Phe 65	
GABA	–3.402	–11.652	–15.054	–6.693	–8.628	–8.315	–23.635
Piracetam	–3.295	–3.771	–7.066	–15.209	–17.001	–8.056	–40.266
PirAc	–3.126	–7.932	–11.058	–18.353	–10.854	–12.668	–41.875
PirPr	–3.387	–7.281	–10.669	–16.311	–12.056	–12.136	–40.504
PirBut	–3.045	–7.515	–10.559	–10.936	–15.734	–10.192	–36.862
Aniracetam	–2.707	–6.371	–9.077	–17.433	–16.844	–9.811	–44.089
Fonturacetam	–0.501	–12.521	–13.022	–23.481	–16.458	–14.223	–54.162
Phenibut	–1.416	–9.197	–10.614	–26.125	–12.417	–11.563	–50.105
Picamilon	–7.555	–3.325	–10.880	–25.993	–10.147	–13.068	–49.207
Pramiracetam	–2.281	–7.117	–9.399	–17.954	–14.522	–10.145	–42.620

Table 4 – Calculated interaction energies of the studied compounds with residues of amino acids Thr 130, Arg 67, Tyr 205, Tyr 157, Arg 207, Glu 155, Phe 200, Thr 202, Tyr 97, Asp 44 и Leu 118, kcal/mol

Amino acid	GABA	Piracetam	PirAc	PirPr	PirBut
Arg 207	0.561	–	–0.590	–2.436	–3.291
Glu 155	–1.881	–1.524	–2.67262	–0.83446	–0.998
Phe200	–5.189	–13.994	–11.515	–10.532	–16.502
Thr 202	–4.137	–5.205	–8.562	–7.454	–8.294
Tyr 97	–1.231	–7.239	–4.117	–5.967	–4.487
Tyr 157	–8.628	–17.001	–10.854	–12.056	–15.734
Tyr 205	–6.693	–15.209	–18.353	–16.311	–10.936
Arg 67	–11.652	–3.771	–7.933	–7.281	–7.515
Asp 44	0.718	–	–	–	–
Leu 118	–1.989	–1.795	–1.951	–1.887	–1.954
Thr 130	–3.40186	–3.295	–3.125	–3.387	–3.045

Table 5 – The minimum value of the interaction energy of ligands with the binding site of the AMPA receptor

Ligand	Minimum energy of ligand-receptor interaction, kcal/mol
Piracetam	–80.3646
PirAc	–94.9684
PirPr	–101.0150
PirBut	–107.0790

Table 6 – Interaction energies of the studied compounds with amino acids of the AMPA receptor binding site, kcal/mol

Amino acids	Piracetam	PirAc	PirPr	PirBut
Asp 248	–8.6384	–7.0767	–7.2909	–7.1325
Leu 247	–17.2258	–10.5279	–10.946	–10.5261
Lys 251	–3.2265	–3.7261	–4.2831	–3.9035
Met 107	–7.8311	–14.4516	–15.6796	–14.981
Phe 106	–2.7516	–11.6726	–12.144	–11.9543
Pro 105	–0.9851	–7.7395	–10.1021	–12.8108
Ser 108	–0.6031	–9.5983	–10.9815	–11.098
Ser 242	–4.3652	–6.5524	–6.1867	–6.4454
Tyr 35	–3.6829	–1.7938	–2.3966	–1.9694
Asp 216	–0.3789	–	–	–
Lys 218	–1.24	–2.8374	–3.687	–6.3321
Ser 217	–7.1949	–11.9722	–11.3445	–12.6389
Sum	–58.124	–87.9485	–95.042	–99.792

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Table 7 – The interaction energy value of ligands from the database bindingdb.org with amino acids Ser 217 and Asp 248 of the binding site of the AMPA receptor

Ligand code according to the database bindingdb.org	K_i, nM	Interaction energy with Ser 217, kcal/mol	Interaction energy with Asp 248, kcal/mol	Total interaction energy, kcal/mol
BDBM50128264	370	–10.4842	–1.3701	–11.8543
BDBM50107595	218	–15.0233	–2.2196	–17.2429
BDBM50126764	175	–13.3183	–5.1289	–18.4472
BDBM50252922	112	–18.9924	–4.4265	–23.4189
BDBM50060627	105	–14.7070	–4.4979	–19.2049
BDBM50252920	80	–17.1936	–2.4128	–19.6064
BDBM50252873	61	–15.8562	–5.0815	–20.9377
BDBM50060632	45	–14.1395	–7.0648	–21.2043
BDBM50060635	4	–14.2827	–7.0615	–21.3442
BDBM50166287	2.9	–20.0334	–6.1673	–26.2007

Table 8 – The predicted value of the biological activity of N-acyl derivatives of 2-oxo-1-pyrrolidine acetamide

Ligand	Interaction energy with Ser 217, kcal/mol	Interaction energy with Asp 248, kcal/ mol	Total interaction energy, kcal/mol	K_i predicted, nM
Piracetam	–7.1949	–8.6384	–15.8333	224.3759
PirAc	–11.9722	–7.0767	–19.0489	140.6609
PirPr	–11.3445	–7.2909	–18.6354	151.4260
PirBut	–12.6389	–7.1325	–19.7714	121.8514

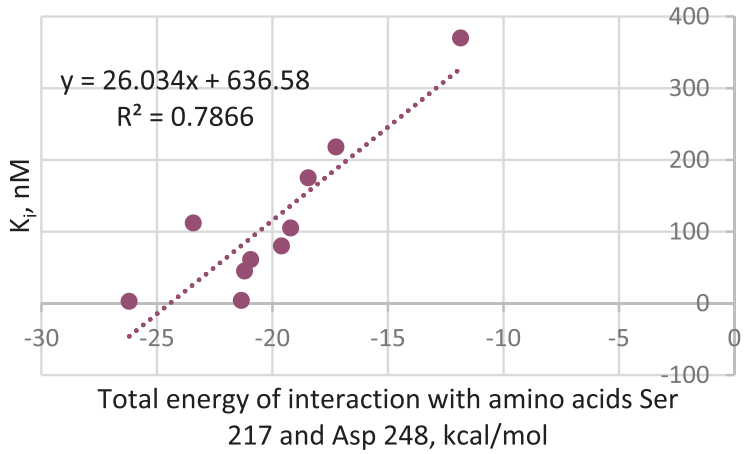

Figure 7 – Precise diagram of the linear relationship between the value of the inhibition constant and the total interaction energy of ligands with amino acids Ser 217 and Asp 248 of the AMPA-receptor binding site

The index of the inhibition constant (K_i) can be used to assess the affinity of low-molecular compounds to the binding site of the protein target. In order to develop a methodology for predicting the biological activity of the studied compounds in relation to the AMPA receptor, 10 structural formulas and their corresponding K_i values for the *Rattus norvegicus* organism were used, they had been given in the Binding Database (https://www.bindingdb.org/bind/index.jsp) (Table 1).

Objects of molecular design

N-acyl-substituted 2-(2-oxopyrrolidine-1-yl)-acetamide (piracetam) were selected as ligands for the molecular design of GABA-ergic and glutamatergic BA**C**s and the subsequent synthesis. They are: N-[2-(2-oxopyrrolidine-1-yl)-acetyl]-acetamide (PirAc), N-[2-(2-oxo-pyrrolidine-1-yl)-acetyl]-propionamide (PirPr) and N-[2-(2-oxo-pyrrolidine-1-yl)-acetyl]-butyramide (PirBut).

The synthesis of N-acyl derivatives of 2-(2-oxopyrolidine-1-yl)-acetamide was carried out by dissolving

a suspension (0.01 mol) of 2-(2-oxopyrrolidine-1-yl) acetamide in the excess of the corresponding anhydride at the temperature of 70–80°C during the stirring. Then 0.1 ml of concentrated sulfuric acid was added. The acylation reaction was monitored by thin-layer chromatography. The target product was isolated from the cooled reaction medium with diethyl ether. Recrystallization of the substance was performed from ethyl or isopropyl alcohol (Fig. 3) [12].

RESULTS AND DISCUSSION

Molecular docking to the $GABA_A$ receptor binding site

The study of the ligand-receptor complex of gamma-aminobutyric acid 6D6U revealed that ligand forms two hydrogen bonds with amino acids Thr 130 and Arg 67, and enters into hydrophobic interactions with Tyr 205, Tyr 157 and Phe 65 in the active center of the $GABA_A$ receptor (Fig. 4).

The results of molecular docking (Table 2) show that all N-acyl derivatives of 2-(2-oxopyrolidine-1-yl) acetamide exceed a number of the known ligands by the value of the minimum interaction energy, such as GABA, piracetam, anipiracetam, picamilonum and pramiracetam. The calculated energies are also comparable to the interaction energy of phenibut, but are inferior to phenylpuracetam (fonturacetam). From the values of the average energy of interaction with the active site of the GABA receptor it follows that PirAc, PirPr and PirBut have the greatest affinity for the GABA receptor among the studied structures.

The further study of the ligand-receptor interaction consisted in comparing the energies of hydrogen bonds and hydrophobic interactions. The energies of hydrogen bonds with amino acids Thr 130 and Arg 67 show that the ligands under study form a more stable hydrogen bond with Arg 67 residue. GABA and phenylpiracetam can form the strongest hydrogen bonds. In terms of the total energy of hydrogen bonds, they are inferior to PirAc, PirPr and PirBut, but all these ligands are superior in energy values to the modified drug – piracetam (Table 3). In other compounds, this energy is comparable to similar energies of N-acyl derivatives of 2-oxo-1-pyrrolidine acetamide. The calculated energies of hydrophobic interactions with amino acid residues Tyr 205, Tyr 157 and Phe 65 show that the simulated structures are characterized by higher interaction energies compared to GABA. However, these values are comparable to those of piracetam, aniracetam and pramipiracetam, but are inferior to fonturacetam, phenibut and picamilon. These results can be explained by the structural feature of phenylpuracetam, phenibut and picamilon, i.e. the presence of volumetric aromatic fragments in their structure. The molecules of phenylpuracetam and phenibut contain a benzoin ring, and picamilonum contains a pyridine heterocyclic system.

The experimental data of the X-ray diffraction analysis of the $GABA_A$-receptor complex with a gamma-aminobutyric acid molecule [6] made it possible to increase the number of amino acid residues involved in the formation of the ligand–receptor complex. Fragments of Arg 207, Glu 155, Phe 200, Thr 202, Tyr 97, Asp 44 and Leu 118 should be added to the previously considered amino acid residues. A comparative analysis of the calculated energy characteristics of binding GABA, piracetam and its modified derivatives (Table 4) suggested that the N-acyl derivatives of 2-oxo-1-pyrrolidinacetamide form a more stable complex with amino acid residues Arg 207, Phe 200, Thr 202, Tyr 97, Tyr 157, Tyr 205 and Phe 65 than the GABA molecule. The interaction energy of the designed structures with the Arg 67 residue is lower compared to GABA, and the results for amino acids Leu 118 and Thr130 are comparable. PirAc has a stronger interaction with the Glu 155 residue than PirPr and PirBut. The amino acid of the active center of the $GABA_A$ receptor Asp 44 does not participate in the interaction with piracetam and its modifications, however, it should be noted that the GABA molecule also forms a weak interaction with it. Thus, PirAc, PirPr and PirBut have a greater affinity for the active center of the GABA receptor compared to the GABA molecule and the drug piracetam.

Molecular docking to the AMPA receptor binding site

According to the results of molecular docking in the Molegro Virtual Docker 6.0.1 program, the most energetically favorable locations of N-acyl derivatives of 2-oxo-1-pyrrolidine acetamide at the binding site of the AMPA receptor were established. Fig. 6 shows examples of the location of the studied compounds with the minimum energy in the interaction with the amino acids of the AMPA-receptor binding site. This figure shows the main amino acids that are involved in the implementation of the activating effect on the receptor: Ser 217, Asp248, Tyr 35 and Asn 252.

The minimum value of the interaction energy of ligands with the binding site of the AMPA receptor is shown in Table 5. These results show that all three N-acyl derivatives of 2-oxo-1-pyrrolidine acetamide have a greater affinity for the binding site of the ionotropic glutamate receptor than the piracetam molecule. At the same time, the most energetically favorable location is occupied by PirBut. Then PirPr and PirAc molecules follow, according to the interaction energy with the binding site of the AMPA receptor.

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Using the 3LSF protein-ligand complex, it was found out that the piracetam molecule, in addition to Ser 217, Asp248, Tyr 35 and Asn 252, also interacts with the following amino acids of the active center of the AMPA receptor: Leu 247, Lys 251, Met 107, Phe 106, Pro 105, Ser 108, Ser 242, Asp 216 and Lys 218. The interaction energies of the piracetam molecule with the amino acids of the AMPA receptor binding site were obtained from the ligand complex 3LSF according to the already established X-ray diffraction analysis of the pharmacologically active location of this substance. In this case, the second location of the piracetam molecule was used, where the formation of bonds with Asn 252 does not occur (Fig. 2A). The interaction energies of PirAc, PirPr and PirBut with the amino acid environment of the AMPA-receptor binding site were obtained using a molecular complex with a minimum energy of the ligand-receptor interaction. The results of the study in Table 6 show, that the N-acyl derivatives of 2-oxo-1-pyrrolidinacetamide exceed the piracetam molecule in terms of the interaction energy with amino acids Met 107, Phe 106, Pro 105, Ser 108, Ser 242, Lys 218 and Ser 217. According to amino acids Asp 248, Lys 251 and Tyr 35, the studied substances have a small difference in the interaction energy compared to the piracetam molecule (the maximum difference in PirAc and Tyr 35, is 1.8891 kcal/mol), and there is a decrease in the interaction energy with the amino acid Leu 247. PirAc, PirPr, PirBut do not interact with the amino acid Asp 216, but the piracetam molecule forms a very weak bond with this amino acid (–0.3789 kcal/mol).

The method for calculating nootropic biological activity by molecular docking using the Molegro Virtual Docker 6.0.1 program, has been developed to predict the biological activity of N-acyl derivatives of 2-oxo-1-pyrrolidinacetamide. Based on the results of molecular docking of ligands given in the database bindingdb.org, the ligand-receptor complex with the lowest MolDock Score interaction energy was selected for each ligand with the AMPA receptor binding site. The energy of bond formation with amino acids Ser 217 and Asp 248 of the binding site of the AMPA receptor responsible for the implementation of the pharmacological action, was studied for the selected locations of low-molecular compounds. The results of the study are shown in Table 7.

In order to determine the relationship between the total interaction energy of ligands with amino acids Ser 217 and Asp 248 of the AMPA-receptor binding site and the corresponding values of the inhibition constant, a dot diagram was constructed. As a result, a linear mathematical relationship between the value of the inhibition constant and the total interaction energy of the selected ligands with the amino acids Ser 217 and Asp 248 of the AMPA receptor binding site was obtained: Y=26,034x+636,58. In this mathematical dependence, the value at Y corresponds to the value of the inhibition constant, and the value of X corresponds to the total interaction energy of the ligands with amino acids Ser 217 and Asp 248 of the AMPA receptor binding site.

To assess the reliability of the obtained mathematical dependence between the value of the inhibition constant and the total interaction energy of the selected ligands with amino acids Ser 217 and Asp 248 of the AMPA-receptor binding site, the approximation reliability factor and the root mean square deviation were calculated. The accuracy coefficient of the approximation was obtained using the Microsoft Excel program, and it is 0.7866. The root mean square deviation was calculated using the formula:

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\Delta K_i^2},$$

where: RMSD is a root mean square deviation; N is the number of ligands; the difference between K_i calculated from the derived linear mathematical dependence and K_i – taken from the database bindingdb.org.

The root mean square deviation is 49.15237. Thus, based on the obtained values of the approximation reliability coefficient and the standard deviation, it is possible to talk about an acceptable accuracy of calculating the linear dependence of the inhibition constant on the total energy of the interaction of ligands with amino acids Ser 217 and Asp 248 of the AMPA-receptor binding site.

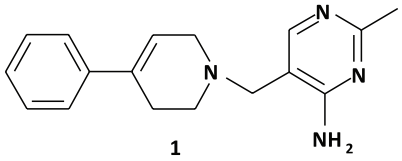
According to the developed methods, the prediction of the biological activity of N-acetyl-2-(2-oxycyclopentyl acetamide)acetamide (PirAc), N-propanoyl-2-(2-oxycyclopentyl)-acetamide (PirPr) and N-butanoyl-2-(2-oxycyclopentyl)- acetamide (PirBut) regarding the AMPA receptor, were calculated (Table 8).

DISCUSSION

The pharmacological effect of nootropic drugs is associated with their effect on the work of GABA-ergic, monoaminergic, cholinergic and glutamatergic neurotransmitter systems of the brain [13].

In addition to the involvement of GABA receptors and metabotropic glutamate receptors (mGluRs) in the nootropic activity, the effect on other G-protein coupled receptors (GPCRs) is also conjugated with the nootropic effect [14]. In particular, the pyrimidine derivative Ro10-5824 (1) shows a pronounced nootropic effect as the agonist of dopamine D4 receptors. The activation of this type of receptors

is associated with an increase in cognitive functions of the brain, such as a learning ability, a cognitive activity, etc. [15].



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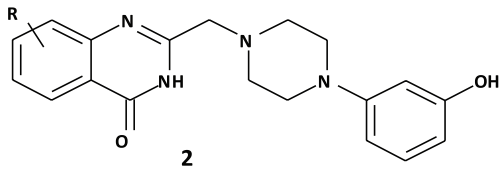
The antioxidant effect of some nootropics is explained by the fact that their molecules are able to inhibit the formation of free radicals and the processes of lipid peroxidation [16].

In [17], using the example of (3H)-quinazoline-4-one derivatives, the possibility of a directed combination of various pharmacophores in one molecule was shown in order to enhance the target pharmacological activity.



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For example, quinazolinone derivatives (2) were synthesized. Their ability to activate brain dopamine D4 receptors is combined with an antioxidant effect due to the presence of a phenolic hydroxyl (a hydroxyphenyl fragment). It was shown [17] that one of the most useful manifestations of such a combination is an increase in the blood flow in the microcirculatory bed and, as a result, an improvement in peripheral blood circulation.

Thus, having the possibility of simultaneous effects on various pharmacological mechanisms, nootropic drugs are an indispensable group in the modern arsenal of drugs used in the treatment of disorders of functioning of the higher nervous system in humans.

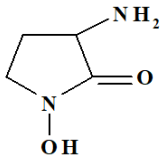
A design of novel therapeutics is based on the structural modifications of the approved drugs, or chemical substances with known activities, including endogenous biologically active compounds. In this regard, close attention is attracted by neuroactive amino acids: GABA, glutamic acid, taurine, etc., which are found to influence a variety of neuronal processes in the brain [18].

A significant role of GABA as an inhibitory neurotransmitter in the relationship between various functions of the central nervous system and the effect on hormonal homeostasis and the activity of the cardiovascular system is shown [19]. It has been proved that the first metabolites of GABA affect the passage of the ketoglutarate dehydrogenase stage of the Krebs cycle.

Accordingly, the GABA-ergic system makes it possible to protect the body in cases of extreme conditions associated with various types of hypoxia by participating in metabolic processes. The influence of GABA on the course of oxidative phosphorylation, the participation in glucose metabolism and, as a result, in the regulation of osmotic processes, and this leads to the manifestation of antihypoxic and antioxidant effects has been confirmed [20].

In the process of searching for biologically active compounds with a nootropic effect, the interaction of the simulated compounds with GABA and NMDA receptors, which are largely responsible for the processes of inhibition and excitation of the central nervous system, is predicted [21]. The optimal relationship between the inhibitory and excitatory neurotransmission systems of the central nervous system ensures the normal activity of the brain, autonomic functions and metabolic processes in the body, and a violation of the balance between these systems leads to various pathological conditions of the body [22]. These facts allow assessing the relevance of the molecular design and targeted synthesis of modified structures of GABA-ergic drugs with cerebroprotective, antihypoxic and nootropic properties.

Cyclic forms of GABA – derivatives of α-pyrrolidone-penetrate through the BBB easier and show an anticonvulsant activity in high doses. The compound 3-amino-1-hydroxypyrrolidinone-2 (3) has a potential for the treatment of the diseases associated with extrapyramidal disorders.



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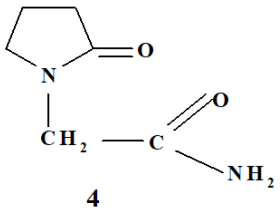

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One of the most successful derivatives of N-substituted lactams synthesized in the laboratory of UCB (Belgium) is 2-(2-oxopyrolidin-1-yl)-acetamide (4). The drug piracetam (4) has a higher lipophilicity than GABA, passes through the BBB easier and affects the cortical, subcortical and transnallosal reactions of the central nervous system [23].

2-(4-hydroxy-2-oxopyrolidine-1-yl) was synthesized as a structural analog of piracetam- acetamide (5), which is a lactam of 4-amino-3-hydroxybutyric acid (the drug gamibetal).

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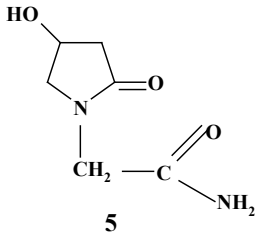

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This drug and its compounds similar in structure are able to normalize memory processes and improve the cognitive properties of the brain [24].

Based on these studies, a new direction has been formed in the field of creating neuropharmacological drugs – a targeted search for gabaergic compounds. At the initial stage, this direction was a set of empirical methods or, at best, it was based on a logical-structural approach. The results of modern studies of X-ray diffraction and radioligand analysis of ligand-receptor complexes sufficiently reliably describe the molecular mechanisms of synaptic processes, which opens up additional opportunities for the molecular construction of GABA-ergic substances in silico, through the use of computer modeling methods [25].

One of the protein targets through which the pharmacological effect of nootropic drugs is realized is the AMPA receptor. This receptor received its name in honor of its selective agonist-α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid). The AMPA receptor is a subtype of ionotropic glutamate receptors that are able to pass positive charged ions through the cell membrane of neurocytes and thus participate in the transmission of rapid excitatory signals in intraneuronal synapses [13].

In these works, special attention of researchers is drawn to the problem of selective interaction of molecules of natural neurotransmitters and ligands with receptors. The emergence of new three-dimensional structures of protein receptors in the Protein Data Bank (www.wwpdb.org) [26], combined with the intensive development of computer methods for analyzing intra-and intermolecular interactions, contribute to the creation of more accurate models in ligand-receptor systems.

The highest GABA-aergic activity can be expected in the molecule N-[2-(2-oxo-pyrrolidine-1-yl)- acetyl]-propionamide. The results of the conducted prognostic study indicate that all N-acyl derivatives of 2-(2-oxopyrolidin-1-yl)-acetamide may be superior in GABA-ergic activity to a modified drug – piracetam, as well as an endogenous inhibitory neurotransmitter – γ-aminobutyric acid.

The analysis of the K_i values obtained in silico for N-acyl derivatives of 2-oxo-1-pyrrolidinacetamide shows that their hypothetical nootropic pharmacological activity significantly exceeds piracetam as a result of their allosteric modulation of the AMPA receptor. In this case, the leader compound is N-butanoyl-2 – (2-oxycyclopentyl)-acetamide. Thus, PirAc, PirPr and PirBut are promising compounds with a higher predicted nootropic pharmacological activity than the piracetam molecule.

The conducted pharmacological studies confirm the pronounced nootropic properties of the synthesized N-acyl derivatives of 2-(2-oxopyrolidine-1-yl)acetamide [27, 28].

CONCLUSION

It was found out that all N-acyl derivatives of 2-oxo-1-pyrrolidinacetamide can surpass gamma-aminobutyric acid and piracetam in a nootropic activity by molecular modeling of the ligands interaction with the active binding site of gamma-aminobutyric acid of the GABA receptor by molecular docking. These compounds have also a great affinity for the binding site of the AMPA receptor. In the course of the conducted studies, a method for predicting glutamatergic activity was proposed.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

I.P. Kodonidi – research design, determination of the structure of the obtained compounds and interpretation of the results of the computational experiment;

A.S. Chiriapkin – synthesis, determination of the structure of the obtained compounds, molecular modeling, analysis of the data of the computational experiment.

D.E. Tworowski – interpretation of the results of the computational experiment. All the authors participated in the discussion of the results and the writing of the article.

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OBITUARY FOR YURI N. CHERNOV
5 Nov 1937 – 1 Jan 2021

On January 1, 2021, Yuri N. Chernov, Doctor of Sciences (Medicine), Professor, Honored Doctor of the Russian Federation, Academician of the International Human Academy in Aerospace Systems, Corresponding Member of the Russian Academy of Natural Sciences, passed away. Yuri N. Chernov was Honorary Doctor of the State Research and Test Institute of Military Medicine of RF Ministry of Defense, a specialist in the field of clinical pharmacology and aerospace radiobiology, Honorary Professor of Voronezh State Medical University n. a. N.N. Burdenko.

Yuri N. Chernov was born on November 5, 1937 in the city of Voronezh. The desire for research work manifested itself already in school years. He was awarded a Certificate of Merit of the Central Committee of the Komsomol (1954), a bronze medal of a participant in VDNKh (Exhibition of Economic Achievements, 1954) for scientific work in biology.

After graduating from Voronezh Medical Institute in 1961 with a degree in General Medicine, he worked as a general practitioner. In January 1964, he took post-graduate courses at the Department of Pharmacology of Voronezh Medical Institute. In 1971 he defended his thesis for the degree of candidate of medical sciences on the topic "The effect of bee venom (apizartron) and its combination with royal jelly (apilak) on the course and outcome of experimental myocarditis". The work was published in five languages and received recognition from the public at the XXIIIrd International Apimondia Apiculture Congress, Moscow (1971).

From 1971 to 1987, Yuri N. Chernov, working as an assistant, a senior teacher, an associate professor of the Department of Pharmacology, combines research, pedagogical work with organizational and social activities. From 1973, he was appointed the deputy dean, from 1992 to 2006, Yuri N. Chernov was three times elected the dean of the medical faculty of Voronezh State Medical Academy.

For 33 years, Yuri N. Chernov worked in the dean's office of the largest medical faculty of VSMU n.a. N.N. Burdenko.

As an outstanding organizer and a scholarly man, Yuri Nikolayevich was able to think strategically, and successfully solved assigned tasks proceeding from the knowledge of human psychology, his own life experience and the ability to carry on a constructive conversation.

Fairness and detachment, honesty and openness, insight, self-control – these were the qualities that Yuri N. enjoyed respect and authority among the teaching staff, graduate students, residents and students for. Among them, there was a catch phrase: "Chernov is more than the dean to the medical faculty, not everyone is born as a dean."

In 1980, by order of the Minister of Defense, Yuri N. Chernov was assigned to the State Research Institute of Aviation and Space Medicine (since 1999, the State Research and Test Institute of Military Medicine of the Ministry of Defence). Under the leadership of Academician of the International Academy of Astronautics (IAA) V.V. Antipov, Yuri N. Chernov dealt with the problems of anti-radiation protection of aircraft crews.

In 1987, Yuri N. headed the Department of Clinical Pharmacology, which was first created, with his active participation, at the Voronezh Medical Academy. He headed the Academy until 2011, and then remained an Honorary Professor of the Department of Clinical Pharmacology in Voronezh State Medical University n. a. N.N. Burdenko. Yuri N. was able to support and encourage

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the colleagues, instill confidence in success and motivate employees to move forward due to being a vivid example of the highest degree of professionalism and great diligence, with tireless energy and committed to his work.

Based on the State Research and Test Institute of Aviation and Space Medicine, in the period from 1980 to 1991, he carried out his thesis research, and in 1991 he defended the doctoral thesis. The topic of his research was "Study of biochemical changes in the brain against the background of functional and behavioral manifestations of radiation damage to the central nervous system and the development of means of pharmacological correction of these disorders."

Professor Chernov, a talented scientist-pharmacologist and a toxicologist, always strived to develop new directions of scientific research necessary for solving problems in clinical practice.

Under the leadership of Yuri N., the following scientific studies were carried out: monitor side effects of drugs; pharmacogenetics and environmental pharmacology, clinical pharmacology of drugs for the treatment of diabetes mellitus; arterial hypertension; peptic ulcer of the stomach and duodenum; drug interactions; antimicrobial therapy; pharmacological correction of endothelial dysfunction.

Professor Chernov attached great importance to the problems of pharmacoeconomics and pharmacoepidemiology, including the development of software for calculating the costs of pharmacotherapy in real clinical practice.

Professor Chernov's scientific research comprised not only clinical works but also works on experimental pharmacology including the following problems: a way to optimize the therapy of poisoning with antipsychotic drugs; search for adaptogenic activity of natural compounds; the study using pharmacological analysis of structural and histochemical changes in the cerebral cortex in cerebral syndrome of acute radiation sickness; pharmacological correction of exposure to ultra-lethal doses of ionizing radiation with the subsequent development of new radioprotectors.

The main scientific studies on the basis of the Institute of Aviation and Space Medicine were carried out in the field of radiobiology, and a comprehensive assessment of the operators' health was also developed to ensure a high level of combat effectiveness and prolong the "career longevity" of the Air Force personnel, as well as the principles of effective and safe prescription of drugs for individuals of camera professions.

Professor Chernov is the author of 520 scientific publications, 5 textbooks on clinical pharmacology, 27 textbooks, 6 monographs, 2 chapters of the national guidelines on clinical pharmacology (2009), practical guidelines on aviation and clinical medicine (2011), 42 patents and 5 software systems, registered in the Register of programs of the Russian Federation.

Professor Chernov created a scientific school of clinical pharmacologists. Yuri N. was a thesis director of 32 theses for a candidate degree, 4 theses for a doctor's degree. Professor Chernov's students successfully work in Russia, Commonwealth of Independent States, Europe, the USA and Great Britain.

Yuri N. Chernov, a wonderful teacher and a brilliant speaker, devoted a lot of effort to teaching clinical pharmacology. His bright lectures had always attracted the interest of students, graduate students, clinical residents, doctors of postgraduate training. Professor Chernov participated in the development of curricula in the discipline for medical universities of the country, in the creation of methodological manuals aimed at the formation of a systematic approach to mastering the necessary professional knowledge among graduate students for the effective and safe use of drugs. The presence of outstanding business and personal qualities helped him in solving numerous problems.

For the first time in the Central Black Earth Region, Professor Chernov organized a course of postgraduate training for doctors of clinical pharmacologists. The result of his efforts was the organization of a clinical pharmacology service in the Voronezh and Lipetsk regions, training of doctors, i.e. clinical pharmacologists for medical organizations in Tambov and Belgorod.

For 5 years, Professor Chernov was a member of the Pharmacological Committee of the USSR (1st Commission); a Member of the Board of the Association of Clinical Pharmacologists of Commonwealth of Independent States; Member of the problem commission No.32.02 of the Russian Academy of Medical Sciences "Pharmacology of heart and blood vessels"; Member of the Bureau of the Problem Commission No.32.06 "Clinical Pharmacology" (First Moscow State Medical University n. a. Sechenov); Member of the Thesis Board of VSMU n. a. N.N. Burdenko.

Professor Chernov was also Member of the editorial board of the journals "Experimental and Clinical Pharmacology" (Moscow), "Medicine remedies" (Moscow), the federal annual guidelines "Formulary system".

Professor Chernov made a scientific contribution to the development and strengthening of international relations of pharmacologists of our country with the European scientific community.

From 1997 to 2018, he actively supported scientific contacts with the Institute of Clinical Pharmacology of the Charite Clinic (Germany), where joint studies were carried out to research the role of polymorphic enzymes in the development of various types of cancer and where, under his leadership, the scientific work "Genotyping of drug enzymes metabolism" was executed.

Professor Chernov was a clinical pharmacologist of the highest medical qualification, an honored doctor of Russia, had the title of "Excellent Worker of Public Health". For achievements in space medicine and numerous discoveries, he was awarded a diploma of the Commander-in-Chief of the Air Force, the Order of

Friendship of Peoples, the Medal of the Air Force Veteran, the Medal n. a. Yu. A. Gagarin, the Order of Merit to the Fatherland, II degree, the medal n. a. G.K. Zhukov, the medal "Veteran of Labor", the merit badge "For Merit to the Voronezh Region". Professor Chernov was also a prize winner of the forum "Golden Fund of the Voronezh Region" in the nomination "Education".

Yuri N. Chernov was a great scientist, a wonderful teacher, a bright personality, a man of great depth and courage. Professor Chernov's high professionalism, wisdom and good-heartedness will always be in the memory of the scientific community, as well as his many students, colleagues and everyone who was happy to know and work with Yuri N.

REST IN PEACE!

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