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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.

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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