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HYPOGLYCEMIC EFFECT OF SITAGLIPTIN AND AMINOGUANIDINE COMBINATION IN EXPERIMENTAL DIABETES MELLITUS

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The aim of the work was to determine the antidiabetic effect of a sitagliptin and aminoguanidine combination in rats with experimental diabetes mellitus.

Materials and methods. The study was carried out on male Wistar rats and C57BL/KsJ-db/db mice. According to the models used, it was divided into 4 series, in which alloxan, steroid-induced (dexamethasone) and streptozotocin-nicotinamide-induced diabetes mellitus (DM) were formed, respectively, in rats, and in the 4 series, obese C57BL/KsJ-db/db mice were used. In the 1 and 2 series, the treatment was started prophylactically – 3 h after the alloxan administration and simultaneously with the dexamethasone administration, in the 3rd and 4th series, the treatment was carried out after the pathology had developed – 7 days after the streptozotocin with nicotinamide administration, and in the obese mice – immediately after their distribution according to the groups. The treatment was carried out with sitagliptin (10 mg/kg), aminoguanidine (25 mg/kg), or a combination thereof. The treatment was continued till the end of the experiment, which was completed with an oral glucose tolerance test (OGTT) after 4 h of fasting. The obtained data were subjected to statistical processing.

Results. In the course of the experiments, it was found out that the prophylactic administration of a sitagliptin and aminoguanidine combination, unlike each of the components, prevented the development of alloxan DM. More effectively than the administration of sitagliptin alone, it reduced the severity of steroid-induced DM, which was expressed in a significantly lower level of fasting glycemia (after 4 h of fasting) and postprandial glycemia (during OGTT). Under the conditions of streptozotocin-nicotinamide-induced DM, the studied combination slowed down the progression of the pathology, and in the obese mice, the course therapeutic administration of sitagliptin and its combination reduced the severity of carbohydrate metabolism disorders (fasting glycemia) and increased the rate of glucose utilization.

Conclusion. As an iNOS blocker, aminoguanidine enhances the antidiabetic effect of sitagliptin, preventing the development of alloxan diabetes and reducing the severity of steroid-induced DM when administered prophylactically. When administered therapeutically, it reduces the severity of streptozotocin-nicotinamide-induced DM in rats and type 2 DM in mice with a predisposition to obesity.

Keywords: DPP-4 inhibitors; sitagliptin; preclinical studies; diabetes; alloxan; streptozotocin

Abbreviations: eNOS – endothelial nitric oxide synthase; iNOS – inducible nitric oxide synthase; nNOS – neuronal nitric oxide synthase; NO – nitric oxide (II); GLP-1 – glucagon-like peptide-1; DPP-4 inhibitors – inhibitors of dipeptidyl peptidase-4; OGTT – oral glucose tolerance test; DM – diabetes mellitus; SDM – steroid-induced diabetes mellitus.

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ГИПОГЛИКЕМИЧЕСКОЕ ДЕЙСТВИЕ КОМБИНАЦИИ СИТАГЛИПТИНА С АМИНОГУАНИДИНОМ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ САХАРНОМ ДИАБЕТЕ

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Цель. Определить эффективность противодиабетического действия комбинации ситаглиптина с аминогуанидином у крыс с экспериментальным сахарным диабетом.

Материалы и методы. Исследование проведено на крысах-самцах линии Wistar и мышах линии C57BL/KsJ-db/db. Согласно используемым моделям, оно было разделено на 4 серии, в которых формировали аллоксановый, стероид-индуцированный (дексаметазоновый) и стрептозотоцин-никотинамид-индуцированный сахарный диабет (СД) у крыс. В 4 серии использовали склонных к ожирению мышей линии C57BL/KsJ-db/db. В 1 и 2 сериях лечение начинали профилактически – через 3 ч после введения аллоксана и одновременно с введением дексаметазона; в 3 и 4 сериях лечение проводили после сформировавшейся патологии – через 7 сут после введения стрептозотоцина с никотинамидом и у мышей с ожирением сразу после их распределения по группам. В качестве лечения вводили ситаглиптин (10 мг/кг), аминогуанидин (25 мг/кг) или их комбинацию. Лечение проводили до конца эксперимента, который завершали пероральным тестом на толерантность к глюкозе (ПТТГ) после 4 ч голодания. Полученные данные подвергались статистической обработке.

Результаты. В ходе проведенных экспериментов было установлено, что профилактическое введение комбинации ситаглиптина с аминогуанидином, в отличие от каждого из компонентов, предотвращало развитие аллоксанового СД, а также более эффективно, чем введение только ситаглиптина снижало выраженность стероид-индуцированного СД, что выражалось в значительно более низком уровне гликемии натощак (через 4 ч голодания) и постпрандиальной гликемии (в ходе проведения ПТТГ). В условиях стрептозотоцин-никотинамид-индуцированного СД исследуемая комбинация замедляла прогрессирование патологии, а у мышей с ожирением терапевтическое курсовое введение ситаглиптина и его комбинации снижало тяжесть нарушения углеводного обмена (уровень гликемии натощак) и увеличивало скорость утилизации глюкозы.

Заключение. Аминогуанидин как блокатор iNOS усиливал противодиабетическое действие ситаглиптина, предотвращая развитие аллоксанового диабета и уменьшая выраженность стероид-индуцированного СД при профилактическом введении, а при лечебном курсовом введении снижал тяжесть течения стрептозотоцин-никотинамид-индуцированного СД у крыс и СД 2 типа у мышей с предрасположенностью к ожирению.

Ключевые слова: ингибиторы ДПП-4; ситаглиптин; доклинические исследования; сахарный диабет; аллоксан; стрептозотоцин

Список сокращений: eNOS – эндотелиальная синтаза оксида азота; iNOS – индуцибельная синтаза оксида азота; nNOS – нейрональная синтаза оксида азота; NO – оксид азота (II); ГПП-1 – глюкагоноподобный пептид-1; иДПП-4 – ингибиторы дипептидилпептидазы-4; ПТТГ – пероральный тест на толерантность к глюкозе; СД – сахарный диабет; ССД – стероид-индуцированный сахарный диабет.

INTRODUCTION

The number of diabetes mellitus (DM) patients registered in Russia at the beginning of 2021, was almost 4.8 million people [1]. While in the world, according to the International Diabetes Federation (IDF), the number of diagnosed and undiagnosed cases exceeded 536 million people in 2021 and, according to its forecasts, by 2045, this number will increase by 46%, reaching 783.2 million people [2]. A low availability of modern hypoglycemic drugs significantly limits the effectiveness of the endocrinological service and medical and social measures aimed at curbing DM and related diseases. Modern guidelines for the treatment of DM indicate the feasibility of an early treatment using rational combinations of drugs, and note the importance of preventing vascular DM complications [3, 4].

Steroid-induced diabetes mellitus (SDM) is also a common and potentially dangerous problem in clinical

practice, affecting almost all medical specialties but often difficult to detect in the clinical setting. Glucocorticoids are widely used as potent anti-inflammatory and immunosuppressive drugs for the treatment of a wide range of diseases. However, they are also associated with a number of side effects, including new onset hyperglycemia in patients without a history of DM or severe uncontrolled hyperglycemia in the patients diagnosed with DM [5]. The mechanism of the steroid-induced diabetes mellitus (SDM) development includes a decrease in insulin sensitivity (respectively, glucose utilization) in target tissues, followed by an increase in catabolic processes (proteolysis and lipolysis) and an increase in the glucose production by the liver (due to the stimulation of gluconeogenesis and glycogenolysis), as well as the suppression of insulin synthesis, including the ones due to the direct damaging effect of steroids on pancreatic β-cells [5].

Currently, scientifically substantiated and reliably tested methods for the prevention of SDM are inconsiderable in number. As with other types of diabetes, the risk factors principles of the early identification and modification are used. SDM screening should be performed in all patients receiving moderate to high doses of glucocorticoids. Problems in the management of SDM are associated with large fluctuations in postprandial hyperglycemia and the lack of well-defined treatment protocols. Along with lifestyle changes, hypoglycemic drugs with an insulin-sensibilizing action are indicated [6, 7]. However, the insulin therapy is often unavoidable, so insulin can be considered the treatment of choice. In the SDM treatment, the degree and nature of hyperglycemia, as well as the type, dose and regimen of glucocorticoids, should be taken into consideration. In addition, it is important to instruct patients and/or their families on how to make the necessary adjustments. Prospective studies are needed to answer the remaining questions regarding SDM. The hyperglycemia that occurs during the use of glucocorticoids, is believed to disappear after their withdrawal, but this is not always confirmed in practice. A reverse situation occurs more often, especially in individuals with risk factors such as obesity or prediabetes, which is confirmed by the data from the patients who have undergone COVID-19 [8].

Dipeptidyl peptidase-4 (DPP-4) inhibitors have a moderate hypoglycemic activity and are often used to create rational combinations of hypoglycemic drugs. The drugs of this pharmacotherapeutic group including the ones created by leading pharmaceutical companies around the world [9] have been developed for more than 30 years. A distinctive feature of the drugs with an incretins activity, which also include DPP-4 inhibitors, is a number of pleiotropic effects associated with a decrease in the risk of developing cardiovascular DM complications [10]. In Russia, DPP-4 inhibitors are actively used in the DM combination therapy. The characteristics of the domestic market for DPP-4 inhibitors are summarized in Fig. 1.

Aminoguanidine is a nitric oxide synthase inhibitor with a high (50-fold) specificity for its inducible isoform (iNOS), as well as an inhibitor of the formation of advanced glycation end products [11]. Under the experimental conditions, the administration of aminoguanidine delayed the formation of autoimmune DM, the rate of plaque formation in a diet with excess cholesterol, and improved the course of alloxan DM [12–14]. A number of studies testify in favor of a significant role of nitric oxide in the development of autoimmune diabetes, and indicate the advisability of using selective iNOS inhibitors to reduce the disease states associated with the nitric oxide expression and its increased production [13–15]. Nitric oxide can be considered as one of the targets for the DM treatment and its complications, since its role in the modulation of insulin secretion and its signaling pathways has been proven [16]. The attention of many researchers is focused on studying the role of iNOS in the pathogenesis of many diseases, including the formation of insulin resistance and death of β-cells in DM. It is known that this enzyme can be induced by many inflammatory cytokines, the increased expression of which accompanies DM and obesity [14, 15, 18].

As a part of the search for means to prevent vascular DM complications, aminoguanidine was previously studied as an antiglycating agent and a drug for the treatment of diabetic nephropathy [19]. However, clinical trials of aminoguanidine were discontinued in phase III due to safety concerns and lack of efficacy. Nevertheless, the therapeutic potential of aminoguanidine is of interest in the development framework of new pathogenetic approaches to the treatment of DM and its complications by creating rational combinations. To do this, it is advisable to study the combination potential of an agent with an incretin activity that improves the function of β-cells and an inhibitor of iNOS aminoguanidine, which is able to reduce the autoaggression of the immune system against β-cells in DM, and has the properties of an antiglycation agent.

THE AIM of the work was to determine the hypoglycemic effect of a sitagliptin and aminoguanidine combination under the conditions of various experimental diabetes mellitus models.

MATERIALS AND METHODS

Model objects

All experiments were performed in accordance with the legislation of the Russian Federation and the technical standards of the Eurasian Economic Union for Good Laboratory Practice (GOST R 53434-2009, GOST R 51000.4-2011). The study design was approved by the Regional Independent Ethics Committee (the registration number: IRB 00005839 IORG 0004900 (OHRP), as evidenced by the extract from Protocol No. 132 dated 20 May 2019 of the meeting of the Commission for Expertise of the Study of the Ethics Committee at Volgograd State Medical University.

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The work was performed on 150 male Wistar rats (aged 6 months, body weight was 300–350 g, Rappolovo Nursery of Laboratory Animals), and 40 male mice of the C57BL/KsJ-db/db line (aged 4–5 months, body weight was 50–60 g), characterized by severe obesity and spontaneously developing severe diabetes, causing necrosis of β-cells, nephro-, neuro-, retinopathy and other complications. The mice of this line carry an autosomal recessive mutation in the leptin receptor gene (linkage group 8, chromosome 4). In the homozygous state, it causes hyperleptinemia, hyperinsulinemia, dyslipidemia, hyperglycemia, obesity and diabetes, which is difficult to correct with the drugs from the DPP-4 inhibitors in monotherapy [20]. As controls, 10 mice of the C57BL/KsJ–db+/+m line were used (*m* gene – misty, a recessive marker of the opposite chromosome that brightens the color and does not carry the db gene); the mice were without obesity and without diabetes (aged 4–5 months, body weight was 20–25 g) [21, 22]. All the mice had been obtained from the nursery of laboratory animals "Stolbovaya" (Scientific Center for Biomedical Technologies of Federal Medical and Biological Agency of Russia). After the arrival from the nursery, the animals were quarantined for 14 days. in the vivarium of Volgograd State Medical University, where they were kept throughout the experiment at 20±2°C under the conditions of 40–60% humidity of an alternating day/night cycle (12/12 h) with an unlimited access to food and water.

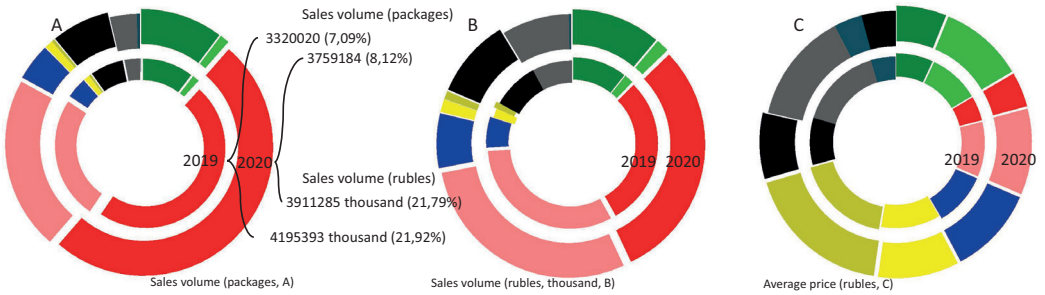
Pathology Modeling

In rats, disturbances in carbohydrate metabolism were induced by multiple intraperitoneal dexamethasone injections (injection solution – 4 mg/ml; KRKA, Slovenia) at the dose of 20 mg/kg/day (within 7 days) or a single intraperitoneal alloxan injection (130 mg/kg; Sigma-Aldrich, USA) [23] or streptozotocin (65 mg/kg, 15 min after 230 mg/kg nicotinamide; Sigma-Aldrich, USA) [24]. Alloxan and streptozotocin were administered to the animals after 36 h of fasting, which provided a better reproducibility of these models due to a decrease in the level of glycemia to the lower limit of the reference range and a decrease in the variability of its level among the animals [24]. The mechanism of the diabetogenic action of these substances is schematically shown in Fig. 2. Due to the ability to selectively interact with the glucose transporter GLUT2, alloxan and streptozotocin accumulate in pancreatic β-cells and have a selective, but different in mechanism, cytotoxic effect. The administration of alloxan leads to the development of an oxidative stress and subsequent death of the β-cells, and causes a state of severe hyperglycemia, which may correspond to type 1 diabetes. In this case, damage to liver and kidney cells is possible due to a slight expression of GLUT2. The cytostatic effect of streptozotocin develops as a result of alkylation of nucleic acids and an increase in the generation of reactive oxygen species. DNA alkylation leads to repair errors, which, when accumulated, lead to the cell death through the activation of apoptotic mechanisms. At the same time, a combined administration of nicotinamide and streptozotocin makes it possible to reduce the activity of PARP-1, which reduces the activity of the SOS repair and somewhat reduces the intensity of apoptosis processes. The administration of streptozotocin and nicotinamide in certain doses (and in certain ratios) makes it possible to achieve a partial decrease in β-cell mass with the development of a moderate hyperglycemia state, which may correspond to one of the forms of type 2 diabetes, characterized by an impaired insulin secretion without insulin resistance [25].

Study design

The overall design of the study is presented below on Figure 3. The study was performed in 4 series: in the first series, the treatment was started 3 h after the alloxan administration, in the second – simultaneously with the first injection of dexamethasone, in the third – after 7 days after the streptozotocin administration, and in the fourth – after quarantine and distribution into groups. Sitagliptin (Januvia, 10 mg/kg/day, *per os*) or aminoguanidine (Sigma, 25 mg/kg/day, i.p.) or their combination, were administered as a treatment (the dose, regimen, and an administration route for each drug remained unchanged). In the 1 and 2 series, in which the treatment had been started before the pathology development, the animals were divided into the groups randomly before the administration of the study drugs. In the 3 series (streptozotocin-nicotinamide-induced DM) and in the 4 series (the obese mice), the level of glycemia was preliminarily measured in the animals with developed pathology. The rats and mice with fasting glycemia levels of more than 11 mmol/l were subjected to the randomization to the experimental groups. The treatment was started immediately after the division into groups. The effectiveness of the therapy was assessed by measuring the concentration of glucose (glucometer Kontur TC, Bayer, Germany) in the blood after 4 h of fasting and/or during an oral glucose tolerance test (OGTT). During that test, the level of glycemia was recorded before and after 60 and 120 min after the oral administration of an aqueous 40% glucose solution at the dose of 4 g/kg, followed by the calculation of the area under the "glycemic level-time" curve ($AUC_{0\text{-}120}$).

In each series of the studies, the animals were divided into equal (n=10) groups: intact, diabetes + placebo (0.9% NaCl) – "placebo", diabetes + sitagliptin – "Sit", diabetes + aminoguanidine – "AMG", diabetes + sitagliptin + aminoguanidine – "Sit + AMG". The doses of the substances were selected taking into account the literature data [19, 26].



	Sales volume (packages, A)		Sales volume (rubles, thousand, B)		Average price (rubles, C)	
Alogliptin	344901 (10,39%)	386596 (10,28%)	415665 (10,63%)	468604 (11,17%)	1090,31 (6,95%)	1027 (6,15%)
Alogliptin + metformin	45844 (1,38%)	43585 (1,16%)	70568 (1,80%)	67604 (1,61%)	1494,12 (9,53%)	1732,54 (10,38%)
Vildagliptin	1584690 (47,73%)	1876337 (49,91%)	1150815 (29,42%)	1267789 (30,22%)	723,23 (4,61%)	719 (4,31%)
Vildagliptin + metformin	824156 (24,82%)	814582 (21,67%)	1259694 (32,21%)	1214527 (28,95%)	1623,7 (10,36%)	1774,82 (10,64%)
Linagliptin	140976 (4,25%)	174160 (4,63%)	235270 (6,02%)	285992 (6,82%)	1576,83 (10,06%)	1799,83 (10,78%)
Saxagliptin	36965 (1,11%)	37853 (1,01%)	69293 (1,77%)	67458 (1,61%)	1754,28 (11,19%)	1669,22 (10,00%)
Saxagliptin + metformin	14636 (0,44%)	14084 (0,37%)	46093 (1,18%)	43739 (1,04%)	2826,98 (18,03%)	3037,6 (18,20%)
Sitagliptin	222278 (6,7%)	272118 (7,24)	357402 (9,14%)	414779 (9,89%)	1395,99 (8,90%)	1355,08 (8,12%)
Sitagliptin + metformin	104762 (3,16%)	126716 (3,37%)	305884 (7,82%)	354919 (8,46%)	2464,31 (15,72%)	2291,16 (13,73%)
Gosogliptin	812 (0,02%)	11675 (0,31%)	599 (0,02%)	8697 (0,21%)	729,67 (4,65%)	578,31 (3,47%)
Evoligliptin	–	1478 (0,04%)	–	1284 (0,03%)	–	703,72 (4,22%)

Figure 1 – Some indicators of iDPP-4 domestic market (according to DSM Group data)[1]

Notes: The data are presented in Russian rubles, dated 1 August 2022, 1 US dollar (USD) corresponded to 61.3 Russian rubles (RUB).

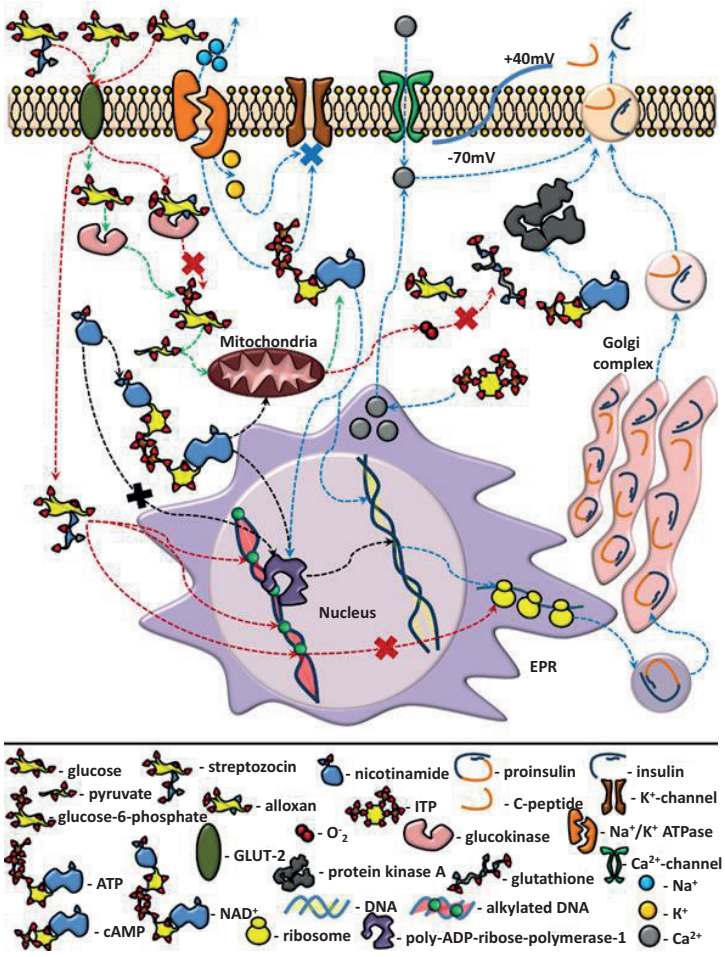


Figure 2 – Mechanism of streptozotocin and alloxan diabetogenic action, adapted from [25]

Note: ADP, adenosine diphosphate; ATP – adenosine triphosphate; GLUT-2, glucose transporter, type 2; DNA – deoxyribonucleic acid; ITP, inositol 1,4,5-triphosphate; mV – millivolt; NAD+, nicotinamide adenine dinucleotide (oxidized); cAMP – cyclic adenosine monophosphate; EPR – endoplasmic reticulum.

[1] The data were officially acquired from the DSM Group company, the calculations were made and the diagrams were presented on their basis.

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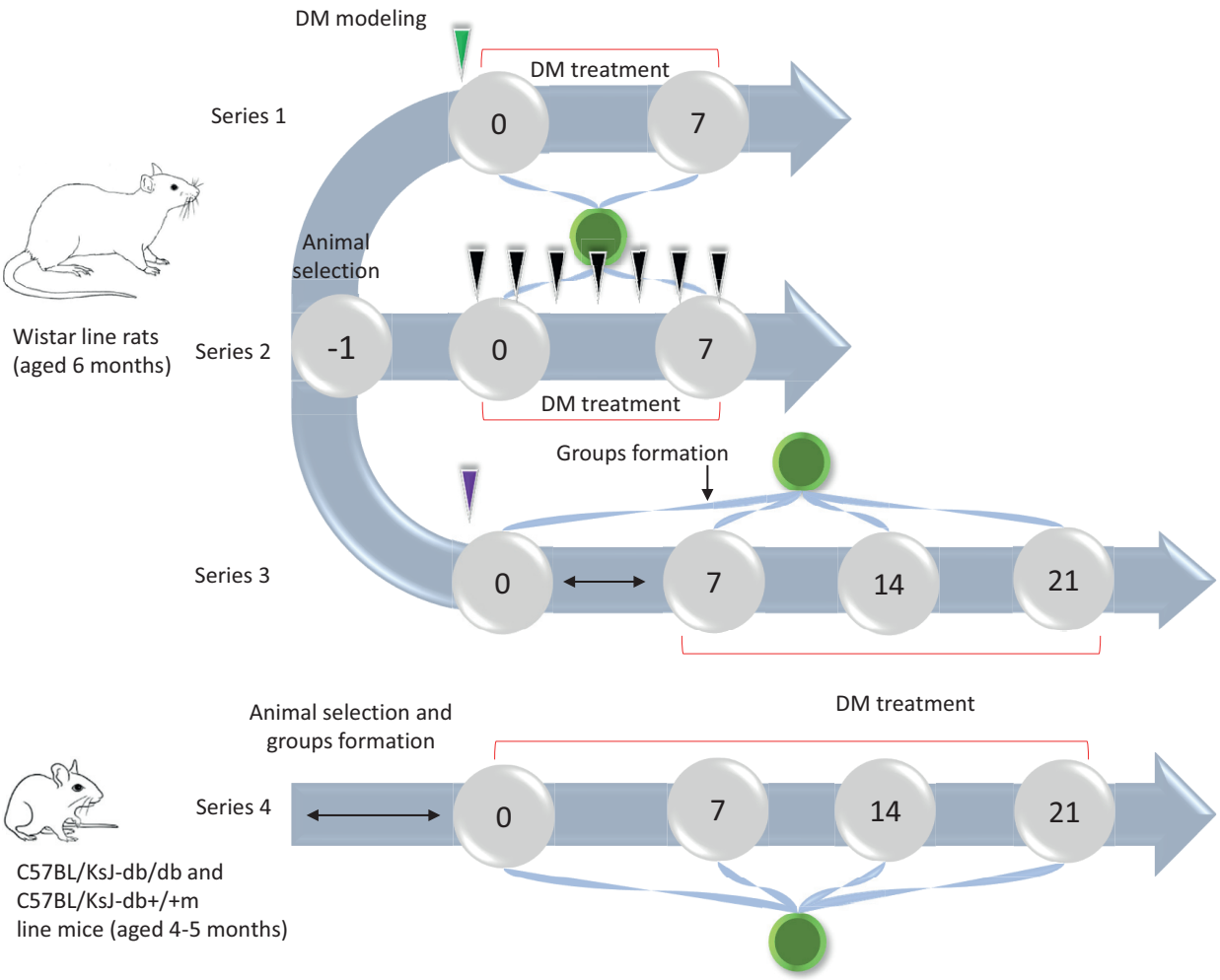

Experimental groups (n=10):
Intact
Diabetes + placebo
Diabetes + Sitagliptin (Sit)
Diabetes + Aminoguanidine (AMG)
Diabetes + Sit + AMG

Determination of glucose in blood
DM treatment
DM development without therapy

Alloxan (130 mg/kg) and test substances
Dexamethasone (20 mg/kg) and test substances
Nicotinamide (230 mg/kg) + streptozotocin (65 mg/kg)

Figure 3 – Study design
Note: AMG – aminoguanidine; DM – diabetes mellitus; Sit – sitagliptin.

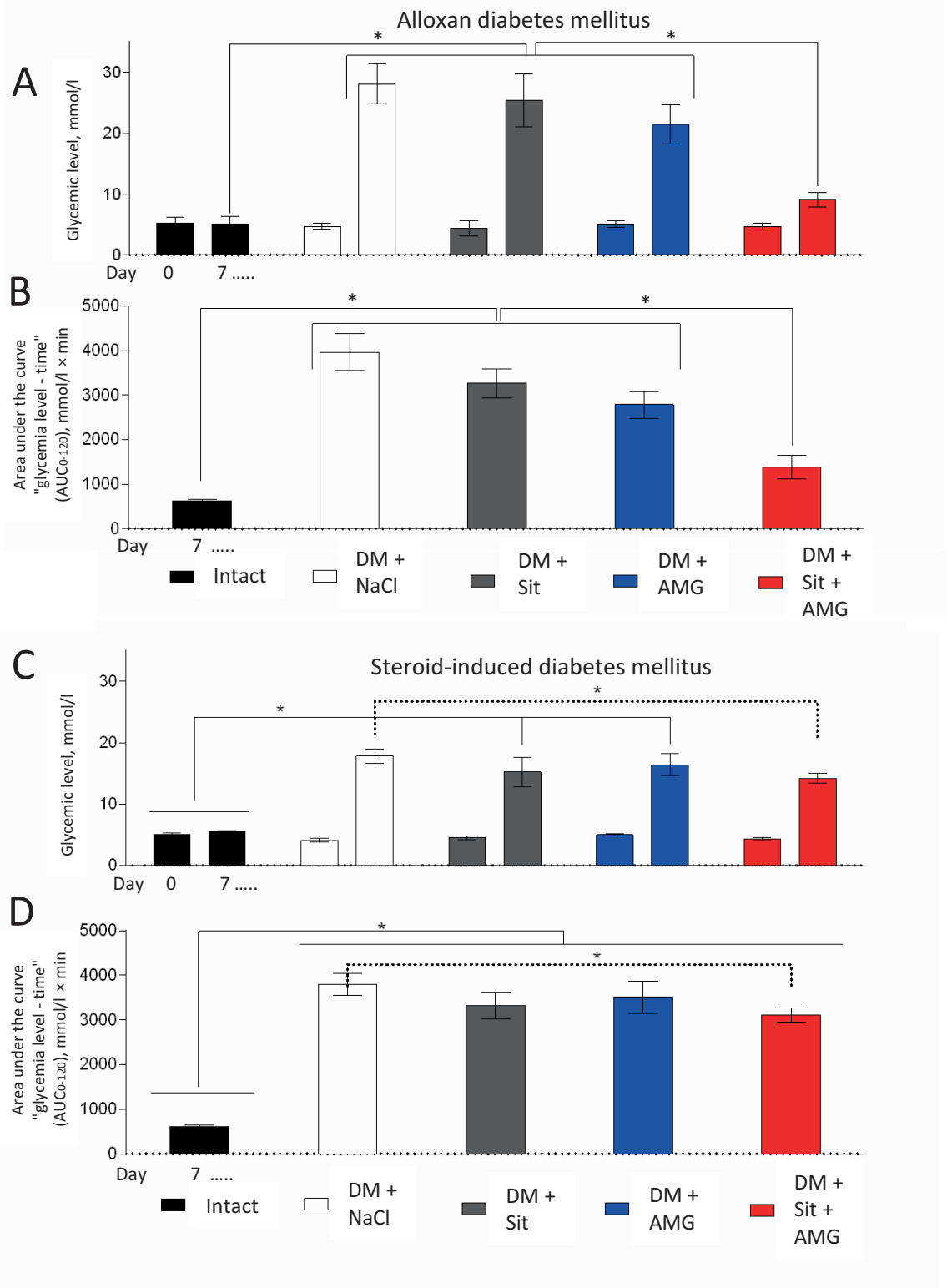

Figure 4 – Influence of sitagliptin, aminoguanidine and their combination in prophylactic administration to rats with alloxan (A, B) and steroid-induced (C, D) DM on fasting blood glucose levels (mmol/l; A, C) and its utilization during oral glucose tolerance test (AUC_{0-120}, mmol/l*min; B, D)

Note: A – the first column – the values before modeling DM (alloxan, day 0), the second – after treatment (day 7); B – area under the curve "glycemic level – time" (AUC_{0-120}) 7 days after treatment of diabetes mellitus caused by the alloxan administration; C – the first column – values before modeling DM (dexamethasone, day 0), second – after treatment (day 7); D – AUC_{0-120} 7 days after treatment for dexamethasone-induced DM; * – $p<0.05$ one-way analysis of variance with Newman-Keuls post-hoc test; compared samples are indicated by lines.

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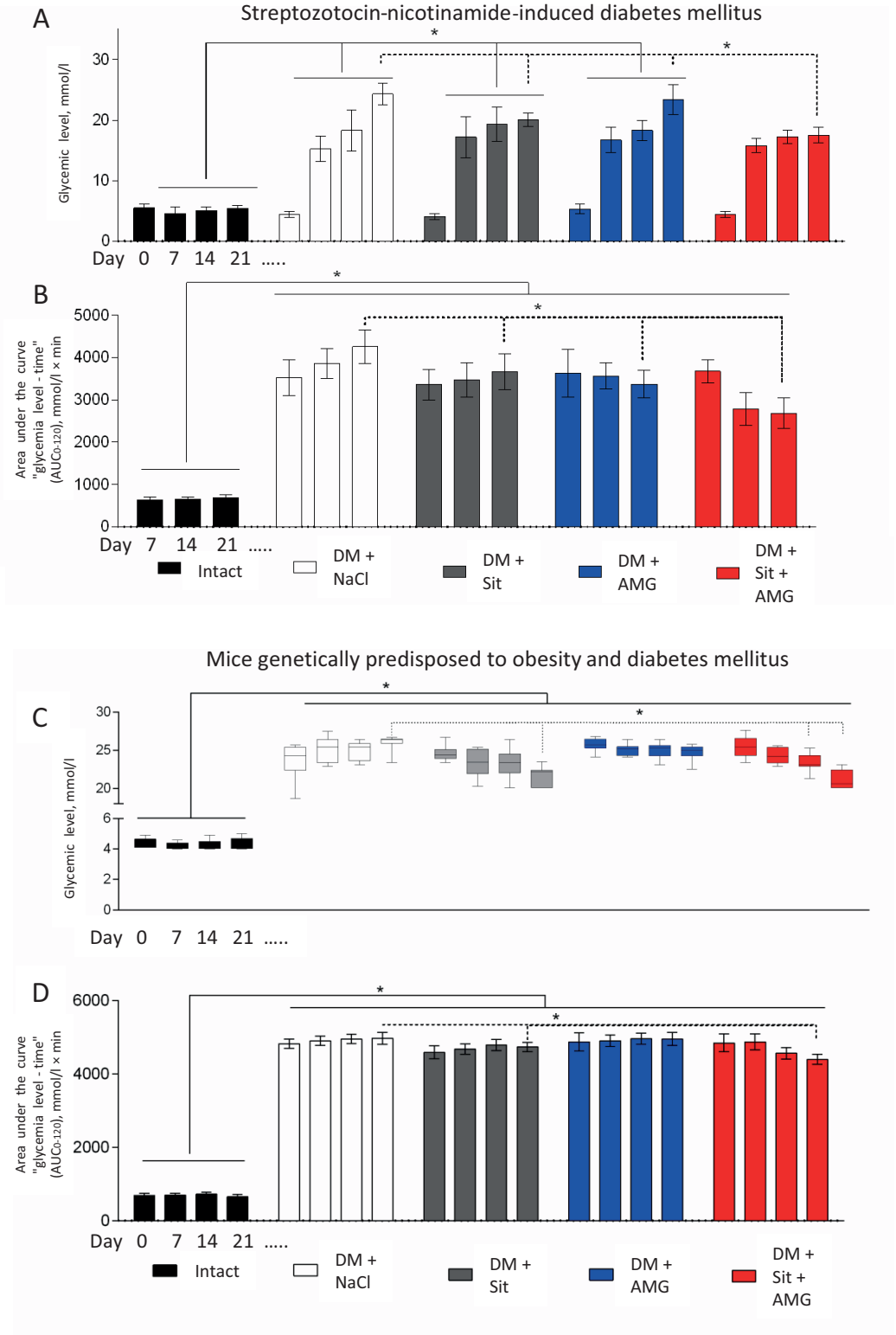

Figure 5 – Effect of sitagliptin, aminoguanidine and their combination in therapeutic administration to rats with streptozotocin-nicotinamide-induced DM (A, B) and in C57BL/KsJ-db/db mice with genetic predisposition to DM (C, D) on glucose levels in fasting blood (mmol/l; A, C) and its utilization during oral glucose tolerance test (AUC_{0-120}, mmol/l*min; B, D) before and after treatment

Note: A – the first column – the values before modeling DM (streptozotocin, day 0), the second – before treatment (day 7), the third and fourth, respectively, after treatment; B – the first column – before treatment (day 7), the second, third columns – AUC_{0-120}, respectively, after treatment; C – the first column – the values before treatment (day 0), the second, third and fourth – days 7, 14 and 21 after treatment, respectively; D – the first column – the values before treatment (day 0), the second, third and fourth – 7, 14 and 21 days, respectively, after treatment; * – $p < 0.05$ one-way analysis of variance with Newman-Keuls post-test; compared samples are indicated by lines.

Statistical processing

Statistical processing of the obtained results was carried out by methods of descriptive and analytical statistics using Prism 6 software (GraphPad Software Inc., USA). The distribution of quantitative indicators was assessed using the Shapiro-Wilk test. Intergroup differences were assessed using a one-way analysis of variance with the Newman-Keuls post-hoc test. The differences were considered significant at $p<0.05$. The numerical values were presented as histograms using the arithmetic mean and a standard error of the arithmetic mean (M+SD), as well as range plots with the median of 25th and 75th percentile.

RESULTS

The alloxan administration causes a pronounced toxic effect on pancreatic β-cells: 7 days after the administration, in the animals that were simultaneously injected with saline (placebo), the concentration of glucose, in the blood was maximum and amounted to 28.1±3.3 mmol/l (the rate of glucose utilization during OGTT in this group was minimal; Fig. 4A and B). This indicates a pronounced violation of carbohydrate metabolism due to the destruction of pancreatic β-cells.

A simultaneous administration of sitagliptin or aminoguanidine with alloxan did not significantly affect the severity of glucose metabolism disorders, the level of hyperglycemia in the animals was 25.4±4.3 mmol/l or 21.5±3.2 mmol/l, respectively. A combined administration of sitagliptin and aminoguanidine had a pronounced protective effect, since in the animals of this group, the glucose concentration was 9.1±1.2 mmol/l (the rate of glucose utilization during OGTT was also significantly higher than in the animals of other groups, $p\leq0.05$; Fig. 4A and B). Thus, a prophylactic administration of a sitagliptin (DPP-4 inhibitors) and aminoguanidine (iNOS inhibitor) combination prevented the development of alloxan diabetes mellitus. This may be due to the combination of the protective incretins action and the inhibitory effect of aminoguanidine against iNOS: an enzyme that, by causing the secretion of a large amount of NO, significantly enhances apoptosis and, accordingly, potentiates the cytotoxic effect of alloxan, and, accordingly, its blockade prevents the occurrence of the oxidative stress and reduces damage to the pancreas β-cells.

In contrast to the mechanism of DM development after the use of alloxan, the course administration of glucocorticosteroids causes an increase in catabolic processes in insulin target tissues, as well as a decrease in the function and death of β-cells. Depending on the administration duration, the initial state of carbohydrate metabolism and other individual characteristics, glucocorticosteroids can cause minor reversible changes, as well as persistent and progressive disorders of carbohydrate metabolism [5]. In the study, a course administration of dexamethasone led to the development of severe hyperglycemia (Fig. 4C and 4D). In the animals treated simultaneously with dexamethasone, sitagliptin (15.2±2.4 mmol/l), aminoguanidine (to a lesser extent, 16.4±1.8 mmol/l) or their combination (to a greater extent, 14.2±0.8 mmol/l, $p<0.05$), the blood glucose concentration was significantly lower than in those who were administrated with dexamethasone and placebo (17.8±1.2 mmol/l).

Like alloxan, the administration of streptozotocin with nicotinamide caused less, but at the same time, pronounced disturbances in glucose metabolism due to incomplete damage to the β-cell mass, which is preferable when conducting the studies lasting more than 14 days, due to less general somatic depletion of the animals. In this series of experiments, the test substances were administered to the animals starting from the 7th day after streptozotocin. In the animals that had been injected with saline (placebo), the glucose concentration increased from 15.2±2.1 (7 days) to 24.3±1.8 mmol/l (21 days), which indicates a progressive destruction of β-cells of the pancreas (Fig. 5A and B). In the animals treated with sitagliptin or aminoguanidine for 14 days after streptozotocin, the glucose concentration increased from 17.2±3.4 and 16.7±2.1 mmol/l to 20.1±1.1 and 23.4±2.5 mmol/l, respectively. A minimal progression of hyperglycemia was notified in the animals that had received a combination of sitagliptin and aminoguanidine for 14 days (from 15.8±1.2 to 17.5±1.3 mmol/l, $p<0.05$), which may also be due to blocking iNOS and reducing the number of factors promoting apoptosis.

The mice on which the last series of studies were carried out, were kept on a standard diet and characterized by extremely high values of hyperglycemia (Fig. 5C and 5D), which is probably a feature of this DM model. The administration of sitagliptin, aminoguanidine or their combination to the animals had a moderate hypoglycemic effect. Glycemic levels in the mice that had been administered with the listed drugs for 3 weeks, was 21.6±1.2 mmol/l, 24.7±0.9 mmol/l, 21.1±1.2 mmol/l, respectively, while in the animals treated with placebo, the concentration of glucose in the blood was 26±0.96 mmol/l.

DISCUSSION

The islet of Langerhans consists of approximately 1000 β-cells, which contain from 10 to 13 thousand granules with 106 insulin molecules each. All NOS isoforms are expressed in pancreatic cells. The production of nitric oxide by inducible synthase (iNOS) significantly exceeds that of other isoforms. Moreover, the iNOS expression is increased at high concentrations

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(≥10 mM) of glucose in the cytoplasm. Nitric oxide is involved in the early phase of insulin secretion, increases the level of cyclic guanosine monophosphate and intracellular Ca^{2+}, promotes insulin synthesis, stimulates the activity of the insulin gene promoter and its expression, increases the blood flow in the pancreas and prevents apoptosis through S-nitrosylation, modulates the dynamic association of glucokinase with secretory granules which, in combination, promotes insulin secretion. In addition to the protective effect of NO at high concentrations, created mainly by iNOS, it has a pronounced negative effect on many body systems, including the insular apparatus.

Physiological (produced by nNOS or eNOS) concentrations of NO, cause positive effects that stop functioning when iNOS is activated and an NO production is too high, which enhances the islet dysfunction. In response to inflammatory stimuli, pancreatic cells increase the iNOS expression, which is accompanied by an increase in NO concentration to cytotoxic levels, causing damage, dysfunction, and death of β-cells, which is important in the onset and progression of DM. The suppression of iNOS has a protective effect on pancreatic cells, which are considered particularly sensitive to damage by free radicals of various origins due to the low level of enzymes of the antioxidant system – superoxide dismutase, catalase, and glutathione peroxidase [16–18].

The activity of inducible NOS is regulated at all stages of the expression, including protein stability, and largely depends on the functional state of the cell. Taking into account the toxicity of excess NO, blockade of iNOS under the conditions of inflammation is a promising experimental approach to control a number of pathological processes. Many attempts have been made to normalize the NOS activity for various diseases: blocking the upstream ROS production, the BH4 administration, the folic acid administration to recycle BH2 to BH4, arginase inhibitors, resveratrol, calcium dobesilate, cavnoxin, NOS transcription enhancers (AVE3085 and AVE9488), L-arginine, blockers and activators of various NOS [11].

Metabolic protection of pancreatic β-cells is a promising approach to the treatment of diabetes mellitus and reduction of its complications. A combination of several drugs with multidirectional effects and, accordingly, influencing various pathogenetic mechanisms is a promising approach in the development of modern drugs. From this perspective, aminoguanidine (an inhibitor of iNOS and an inhibitor of glycation end products formation [19]) and sitagliptin (which increases the level of endogenous incretins that have a protective effect on β-cells [27]) are reasonable candidates for the consciousness of a combined antidiabetic drug able of protecting β-cells and slowing down the progression of diabetes of various etiologies.

In this study, an attempt to evaluate the antidiabetic effect of the combination of the common drug sitagliptin and an agent that exhibits a pronounced iNOS inhibitory effect was made. The models used in this work are characterized by different mechanisms of induction of carbohydrate disorders: complete or partial death of β-cells due to a specific toxin, the formation of steroid-induced DM, and the genetic model of DM2. With respect to the pathology formation moment, the time of therapy initiation differed: a prophylactic administration (alloxan DM with the induction of β-cells complete death and steroid-induced DM with a complex disorder of carbohydrate metabolism) and a therapeutic administration (streptozotocin-nicotinamide-induced DM and a genetic model of DM2), which are characterized, respectively, by the death of most β-cells, and obesity with the primary insulin resistance).

In the course of the work, it was found out that the protective effect of the combination significantly exceeds the effects of individual substances. This fact indicates the synergy of the components, which, in turn, can be explained, among other things, by the diversity of their action. The therapeutic effect was especially pronounced during the prophylactic administration in the model of alloxan DM, which is characterized by a complete death of β-cells, which obviously did not happen against the background of the administration of an iNOS inhibitor and an incretin mimetic (iDPP-4) combination. The protective effect of aminoguanidine on the alloxan diabetes model has been previously stated in the literature, herewith, its prophylactic administration and monotherapy were focused. However, for the development of a pronounced effect, its administration was required for 6 weeks, while the gradually developing protective effect is associated with the restoration of the antioxidant defense system, inhibition of the formation of advanced glycation end products, the reduction of inflammation and restoration of islets [14]. In the current study, only in the group treated with the combination (sitagliptin+aminoguanidine, but not the individual components), the level of glycemia compared with the negative control group was significantly (several times) lower, as early as 1 week after the administration of alloxan. That indicates a protective effect of the combinations in relation to a cellular toxin, which realizes its action through an oxidative stress with a subsequent death of β-cells. Given the nature of experimental diabetes induced by the administration of cytotoxic substances similar in the mechanism of action (alloxan and streptozotocin), i.e., the key role of apoptosis in damage to β-cells and the participation of iNOS in this

process, it can be assumed that the hypoglycemic and protective effect on β-cells in sitagliptin (realizing its action mainly through incretins) is complemented by the anti-apoptotic (antioxidant and anti-inflammatory due to iNOS inhibition) effect of aminoguanidine, which leads to a decrease in the intensification of apoptosis and, accordingly, a lower loss of β-cells. To elucidate the exact mechanism of the combination effect on the β-cell death process, additional research is required to identify the expression of factors that affect apoptosis (e.g., transcription factors, cytokines, effectors and regulators of apoptosis, etc.).

In the second series of the studies, it was found out that the combination of sitagliptin and aminoguanidine simultaneously (prophylactically) administrated with dexamethasone, significantly limits the hyperglycemic effect of the latter (progression of carbohydrate metabolism disorders). The revealed effect was less pronounced compared to the first series, which is possibly due to the complex effect of glucocorticosteroids on carbohydrate metabolism, with a limited number of application points of the studied combination, primarily the protection of β-cells. However, this approach may be of interest for the correction of persistent carbohydrate metabolism disorders after the withdrawal of glucocorticosteroids.

In the third series of the experiments, the therapeutic administration of a sitagliptin and aminoguanidine combination managed to significantly slow down the progression of streptozotocin-nicotinamide DM. That can be also associated with a decrease in the intensity of apoptosis and an improvement in the functional state of the remaining β-cells due to the combination of the hypoglycemic and protective effects of incretins (DPP-4 inhibitors) and the anti-inflammatory effect of aminoguanidine.

In the animals with obesity and DM, a certain hypoglycemic effect of sitagliptin and its combination with aminoguanidine, was also notified. It is known that obesity and insulin resistance cause a cascade of reactions, the common link of which is systemic inflammation with an increase in the secretion of pro-inflammatory cytokines. That accelerates damage to β-cells and the transformation of type 2 diabetes into type 1. The use of aminoguanidine in combination with sitagliptin can significantly affect various pathogenetic links initiated by hyperglycemia, in particular, reduce the level of systemic inflammation by inhibiting the activity of iNOS and its role in the destruction of β-cells. In the further work with this model, due to the severity of the pathology, it is advisable to use more effective incretin mimetics – agonists of the glucagon-like peptide-1 receptor.

CONCLUSION

A combined application of sitagliptin and aminoguanidine increases the antidiabetic effect of individual components, preventing the development of diabetes mellitus after the prophylactic alloxan administration, reducing the severity of carbohydrate disorders after the course administration of dexamethasone. When administered medically, it slows down the progression of streptozotocin-nicotinamide DM and reduces the level of glycemia in the animals with a genetic predisposition to diabetes mellitus and obesity. The sitagliptin and aminoguanidine combination can become a basis for the development of a new promising approach to the treatment of diabetes mellitus and its complications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Denis V. Kurkin, Andrey V. Strygin – study idea and planning, graphic material design, approval of manuscript final version; Tamara M. Andriashvili, Alina A. Sokolova, Nikita S. Bolokhov, Vladislav E. Pustynnikov, Evgeny A. Fomichev – pathology modeling, experimental work; Evgeny I. Morkovin – statistical data processing, graphic material design, text editing; Dmitry A. Bakulin, Yuliya V. Gorbunova – literature data collection and analysis, manuscript writing.

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