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Cerebroprotective effect of sitagliptin and aminoguanidine combination in disorders of cerebral circulation

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The aim of the study was to evaluate a cerebroprotective activity of the sitagliptin and aminoguanidine combination in rats with an acute and chronic cerebral circulation insufficiency, as well as with a traumatic brain injury.

Materials and methods. The study was carried out on male Wistar rats in 3 stages using, respectively, a model of a chronic pathology: a chronic cerebral circulation insufficiency (CCCI), as well as 2 models of the acute brain injury (BI): an acute cerebral circulation insufficiency (ACCI), and a traumatic brain injury (TBI). A CCCI was modeled by a bilateral stenosis of the common carotid arteries (by 50%), a model of a hemorrhagic stroke caused by an intracerebral injection of the autologous blood was used as a stroke, a TBI was modeled by a mechanical damage to the brain tissue. To assess the pathology course severity, the following tests were used: Adhesion test, Open field, Morris water maze test, as well as Garcia and Combs&D'Alecy scales. In the animals with an acute damage to the brain at the end of the experiment, the severity of edema of the affected hemisphere was also determined. The treatment was with sitagliptin (10 mg/kg), aminoguanidine (25 mg/kg), or a combination thereof. The obtained data were subjected to the statistical processing.

Results. In the course of the study, it was found out that the administration of a sitagliptin and aminoguanidine combination, unlike each of the components, had a cerebroprotective effect in the animals with a chronic or acute damage to the brain, reducing the severity of psychoneurological (cognitive and sensory-motor) disorders, as well as the brain edema.

Conclusion. Aminoguanidine, as an iNOS blocker, enhances the action of sitagliptin, preventing the brain edema development and reducing the neurological deficit severity (the severity of cognitive and sensory-motor impairments) in the animals with an acute and chronic cerebral circulation insufficiency**.**

Keywords: DPP-4 inhibitors; sitagliptin; preclinical studies; acute and chronic cerebral circulation insufficiency; traumatic brain injury

Abbreviations: iNOS – inducible nitric oxide synthase; mNSS – modified Neurological Severity Score; NO – nitric oxide (II); HS – hemorrhagic stroke; GLP-1 – glucagon-like peptide-1; iDPP-4 – inhibitors of dipeptidyl peptidase-4; SGLT-2 – sodium-glucose linked transporter-2; BP – blood pressure; CVDs – cerebrovascular disorders; ACCI – acute cerebral circulation insufficiency; CCAs – common carotid arteries; CCCI – chronic cerebral circulation insufficiency; TBI – traumatic brain injury; ED – endothelial dysfunction.

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Церебропротекторное действие комбинации ситаглиптина с аминогуанидином при нарушениях мозгового кровообращения

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Цель. Оценить церебропротекторную активность комбинации ситаглиптина с аминогуанидином у крыс с острым и хроническим нарушением мозгового кровообращения, а также черепно-мозговой травмой.

Материалы и методы. Исследование проведено на крысах-самцах линии Wistar в 3 этапа с использованием модели хронической патологии: хроническое нарушение мозгового кровообращения (ХНМК), а также 2-х моделей острого повреждения головного мозга (ГМ) – острое нарушение мозгового кровообращения (ОНМК) и черепно-мозговая травма (ЧМТ). ХНМК моделировали двусторонним стенозированием общих сонных артерий (на 50%); в качестве ОНМК использовали модель геморрагического инсульта, вызванного внутримозговой инъекцией аутокрови; ЧМТ моделировали механическим повреждением ткани ГМ. Для оценки тяжести течения патологии использовали следующие тесты: «Адгезивный тест», «Открытое поле», «Водный лабиринт Морриса», а также шкалы Garcia и «Combs&D'Alecy». У животных с острым повреждением ГМ в конце эксперимента определяли выраженность отека пораженной гемисферы. В качестве лечения вводили ситаглиптин (10 мг/кг), аминогуанидин (25 мг/кг) или их комбинацию. Полученные данные подвергались статистической обработке.

Результаты. В ходе исследования было установлено, что введение комбинации ситаглиптина с аминогуанидином, в отличие от действия каждого из компонентов по отдельности, оказывало церебропротекторное действие у животных с хроническим или острым вариантом повреждения ГМ, снижая выраженность психоневрологических (когнитивных и сенсорно-моторных) нарушений, а также отёка ГМ.

Заключение. Аминогуанидин как блокатор iNOS усиливает действие ситаглиптина, предотвращая развитие отёка ГМ и уменьшая выраженность неврологического дефицита (выраженность когнитивных и сенсорно-моторных нарушений), у животных с острой или хронической недостаточностью мозгового кровообращения.

Ключевые слова: ингибиторы ДПП-4; ситаглиптин; доклинические исследования; острые и хронические нарушения мозгового кровообращения; черепно-мозговая травма

Список сокращений: iNOS – индуцибельная синтаза оксида азота; mNSS – шкала оценки тяжести неврологической симптоматики; NO – оксид азота (II); ГИ – геморрагический инсульт; ГМ – головной мозг; ГПП-1 – глюкагоноподобный пептид-1; иДПП-4 – ингибиторы дипептидилпептидазы-4; НГЛТ-2 – натрий-глюкозный котранспортер 2-го типа; НМК – нарушения мозгового кровообращения; АД – артериальное давление; ОНМК – острое нарушение мозгового кровообращения; ОСА – общая сонная артерия; ХНМК – хроническое нарушение мозгового кровообращения; ЧМТ – черепно-мозговая травма; ЭД – эндотелиальная дисфункция.

INTRODUCTION

In the Russian Federation, a number of patients suffering from a chronic cerebral circulation insufficiency (CCCI) reaches at least 700 cases per 100 000 people. The CCCI manifests itself as a diffuse and gradually increasing deterioration in the blood supply to the brain tissue, which contributes to the formation of cognitive and sensory-motor disorders. The pharmacotherapy of CCCI is aimed at improving hemoperphysis and metabolism of the nervous tissue, as well as reducing the inflammation [1]. Various types of an acute cerebral circulation insufficiency (ACCI) are widely spread. They are characterized by a pronounced neurological deficit, a high mortality, and require a long-term rehabilitation with the possibility of using neuroprotective drugs with a polytargeted mechanism of action (anti-inflammatory, antioxidant, vasodilating, etc.).

In 2010 and 2015, there were 5.406 and 6.240 million stroke deaths worldwide, respectively. At the same time, a significant mortality – about 60% – is caused by a hemorrhagic stroke (HS), which makes up 10-15% of all types of a cerebral circulation insufficiency (CCI) [2, 3]. Its most significant risk factors are a history of CCI, a high blood pressure (BP), and diabetes mellitus (DM) [4]. The problem of a traumatic brain injury (TBI) is also a serious challenge for modern medicine, especially given a limited number of available and effective treatments that take into account pathogenetic mechanisms (inflammation, edema, etc.) [5].

The consequence of TBI, as well as that of acute acute CCI (ACCI) and chronic CCI (CCCI), is the formation of neurological disorders, different in spectrum and severity, leading to a director disability and a decrease in the quality of life, i.e. a cognitive impairment (problems with memory, attention and learning), sensory, motor and other disorders. DM, which is pandemic in nature, significantly complicates the course severity and worsens the prognosis of acute and chronic brain pathologies associated with both a CCI and TBI [4, 6]. In this regard, there is a need for drugs with a cerebroprotective potential that can improve the course prognosis of various types of brain injuries, which can also be used in DM.

Currently, a wide range of drugs that can improve a person's intellectual and mental abilities to varying degrees, including cases of the brain tissue damage, is available. However, their effectiveness does not fully meet the expectations of specialists, therefore, the search for new compounds and approaches, the use of which would reduce the incidence of dementia, increase the resistance of brain tissues to various adverse factors, facilitate and/or accelerate their recovery after various types of traumatic brain injuries, continues.

Over the past 10 years, the interest of specialists in drugs from the group of incretin mimetics has not decreased. The clinical data indicate a reduction in the risk of occurrence and severity of cardiovascular complications in the patients with type 2 diabetes (myocardial infarction and a stroke) when using primarily analogues of glucagon-like peptide-1 (GLP-1). The open pleiotropic properties of incretin mimetics are only partially associated with a hypoglycemic effect, and their basis is multidirectional effects due to the wide prevalence of the GLP-1 receptor in the body and, accordingly, the consequences of its activation [7, 8]. Dipeptidyl peptidase-4 (iDPP-4) inhibitors, having a moderate antihyperglycemic activity, are ones of the most affordable and sought-after drugs from the group of incretin mimetics, and are being actively studied in the form of new fixed combinations [9].

Aminoguanidine is an inhibitor of the end products glycation formation and an inducible isoform of nitric oxide synthase (iNOS), the activity of which is accompanied by a number of positive effects, but mainly it underlies the pathophysiological processes that make up the essence of most cardiovascular diseases [10]. Given a significant negative role of iNOS in the pathogenesis of a CCI, a decrease in the activity of this enzyme can be an effective way to treat this group of pathologies. Many experimental studies have shown a pronounced protective effect of aminoguanidine [11–14] in various CCIs, accompanied by an iNOS activation, tissue hypoxia and edema.

Initially, aminoguanidine was developed as an antiglycation agent with a target positioning for use in diabetic nephropathy [15]. Despite the termination of the clinical studies due to doubts about their safety and lack of efficacy, the potential of aminoguanidine as a promising molecule for the prevention and treatment of DM and its vascular complications, including combinations with other antihyperglycemic agents, remains undiscovered. As indicated above, aminoguanidine showed a pronounced cerebroprotective effect in various experimental CCI models, cardio- and cerebroprotective potential, not associated with their hypoglycemic effect, was also proven for iDPP-4. Based on this, it seems appropriate to evaluate the possibility of their combined use in preclinical studies in order to correct the consequences of a CCI and in conditions of an acute TBI.

THE AIM of the study was to evaluate a cerebroprotective activity of the sitagliptin and aminoguanidine combination in rats with an acute and chronic CCI, as well as with a TBI.

MATERIALS AND METHODS

Model objects

All experiments were performed in accordance with the legislation of the Russian Federation (GOST R 53434-2009, GOST R 51000.4-2011) and the technical standards of the Eurasian Economic Union for good laboratory practice. The design of the study was approved by the Regional Independent Ethics

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Committee, the registration number IRB 00005839 IORG 0004900 (OHRP), as evidenced by an extract from Protocol No. 132 dated May 20, 2019 of the meeting of the Commission for Expertise of the Study at Volgograd State Medical University.

The work was performed on 150 male Wistar rats (aged 5–6 months, body weight of 300 g, the "Rappolovo" nursery for laboratory animals). 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 conditions of 40–60% humidity and an alternating day/night cycle (12/12 h) with an unlimited access to food and water.

All surgical procedures were performed using combined anesthesia: zoletil 20 mg/kg (Zoletil®100, Valdepharm, France) + xylazil 8 mg/kg (Xyla, Interchemie, Netherlands), intraperitoneally. The eyes of the animals were closed during the operation, and blepharogel was applied to them to prevent the cornea from drying out. After the operation, the wounds were irrigated with a solution of chlorhexidine (0.05%); the edges were sutured with a continuous surgical suture.

Pathology modeling

CCCI simulation by stenosis of common carotid arteries [16]

After isolating the common carotid arteries (CCA), the vessels were tied to a nylon thread of a given diameter, and then it was removed so that the vessel could fill the vacated space, which would allow the blood flow to be restored by only 50%. The blood flow velocity was assessed using dopplerography polygraph MP150 with an LDF100C module (Biopac Systems, USA).

Modeling of hemorrhagic stroke by intracerebral injection of autologous blood [17]

An anesthetized animal was fixed in a stereotaxic apparatus (Shenzhen RWD Life Science Co, China) using a tooth holder and ear fixators. The wool cover was shaved off over the parietal bone. After processing the surgical field with a scalpel, a skin incision along the midline above the bregma (8–10 mm long) was made. Using a RESTAR-03 drill (RESTAR, Russia), a cranial foramen 1 mm in diameter was drilled at the level of the bregma (3.5 mm lateral to the midline) in the left coronal suture. Next, 100 µl of blood was taken from the tail vein into a tuberculin syringe without anticoagulant, fixed in a stereotaxic apparatus, and slowly injected through the cranial foramen under the brain membranes using a 29G needle.

Modeling of traumatic brain injury

To simulate an acute TBI, after a similar preparation of the surgical field, according to the stereotaxic coordinates, a hole was drilled in the parietal bone (ø 4 mm) using a drill, after which the brain tissue was injured with a modified mandrin knife.

Determination of pathological changes severity and treatment effectiveness

A neurological deficit in the animals with an acute pathology (a stroke and a traumatic brain injury) was assessed using the following scales: the 9-point "Combs&D'Alecy" scale and the Garcia scale [18, 19]. The "Combs&D'Alecy" scale was used to detect extrapyramidal disorders, including tests to assess a muscle strength, a tenacity-traction and the animals' balance. A lower total score corresponds to more pronounced neurological disorders. According to the Garcia scale, the severity of the following indicators was determined: a muscle tone, a motor activity, basic physiological reflexes, a coordination of movements, and a tactile sensitivity. For each test, the following scores were assigned: 3 points – normal, 2 points – a slight violation of symmetry, 1–0 points – pronounced violations or a lack of movements.

A psychoneurological deficit in the CCCI animals was assessed using the modified Neurological Severity Score (mNSS) [18]. This scale is scored on a variety of parameters, including a muscle strength, motor skills, a sensory function, and a motor coordination. The maximum number of points is 14, the minimum is 0. The higher the total score on the mNSS scale is the severer the neurological deficit is considered.

Sensory-motor disorders (sensitivity and fine motor skills of the forelimbs) were assessed using an Adhesive test [18, 20]. As a foreign object, square pieces of the adhesive tape (5 mm^2, Veropharm, Russia) were quickly glued to the volar surface of the animal' forelimbs. The severity of sensory-motor disorders was judged by the time of detection and removal of the adhesive plaster from the paws by the animals.

Motor disorders were assessed in the Open field test, in which a spontaneous motor activity was judged by the number of sectors crossed during 3 min, and an orienting-exploratory activity was judged by the sum of the stands and peeps into the holes.

The hydration degree of the brain tissues was determined as follows: the cerebral hemispheres were dissected into ipsi- and contralateral (left and right) parts, the affected hemisphere was weighed and dried in a thermostat at 100°C for 24 h to determine the water content procentage according to the formula:

$$W = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100\%$$

The cognitive functions of the CCCI animals were evaluated in the Morris Water Maze test [18, 21], which is designed to study the formation of the spatial memory. The installation is a water pool, divided into 8 designated zones (4 main and 4 additional; Fig. 1), in the center of one of which there is a platform submerged 1 cm into the water, and on the walls of the installation (at points "N", " E", "S", "W") visual landmarks are fixed.

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The scheme of the experiment is the following: according to the certain scheme, during 4 training days (4 attempts per day), an animal was placed in the water at the distance of 10 cm from the maze wall and the latent period of the flooded platform was recorded (Fig. 1). The test sessions lasted for 1 min with a break between the sets of 30 sec. If the rat did not find the platform within 1 min, it was lured to the platform and left on it for 15 sec.

Study design

The study was performed in 3 series (Fig. 2): in the 1st series, CCCI was modeled by stenosis of the CCAs by 50%, the treatment was started 40 days after the operation and continued for 21 days. In the 2nd and 3nd series of the experiment, the animals were simulated an acute brain injury by the methods of creating TBI or a HS; the treatment was started on the 2nd day after the operation and carried out for 14 days; the severity of symptoms of a neuropsychiatric deficit in all the series was assessed before and after the treatment. Sitagliptin (Januvia, 10 mg/kg/day, *per os*) or aminoguanidine (Sigma, 25 mg/kg/day, i.p.) or their combination was administered as a treatment (dose, regimen, and route of administration for each drug remained unchanged). At the end of the study, the severity of cognitive impairment in the Morris Water Maze test was determined in the CCCI animals, and the severity of brain edema was determined in the animals with an ACCI – after euthanasia.

In each series of the studies, the animals were divided into equal (n=10) groups: intact, ACCI/CCCI+ placebo (0.9% NaCl) – "Placebo", ACCI/CCCI+ sitagliptin – "Sit", ACCI/CCCI+aminoguanidine – "Amg", ACCI/CCCI+sitagliptin+aminoguanidine – "Sit+Amg". The doses of the substances were selected taking into account the literature data [22, 23].

Euthanasia

In accordance with Directive 2010/63/EU of the European Parliament and the Council of the European Union on the protection of animals used for scientific purposes dated September 22, 2010, the animals were euthanized after the completion of the experiment by placing them in a CO_2 chamber.

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. The 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 the arithmetic mean and the standard error of the arithmetic mean (Me+m).

RESULTS

A partial ligation of the CCAs by 50% caused pronounced symptoms of a psycho-neurological deficit in the animals, which manifested themselves on the 61st day of the experiment in the form of an increase in the neurological deficit score on the mNSS scale (4.2±2.1 *vs* 0.1±0.1; p <0.05), a motor decrease (18.6±7.1 *vs* 49.8±15.5; p <0.05), and an exploratory activity (3.1±0.5 *vs* 7.5±1 .6; p <0.05), the latent period of detection and removal of foreign objects from the palms of the animals forelimbs. In these animals, cognitive impairments have been also noted, since they memorized the location of the flooded platform in the Morris Water maze test much longer (54.9±12.9 *vs* 22.1±7.2; p <0.05, respectively). The animals with partially ligated OSAs and administered with sitagliptin or aminoguanidine for 21 days, had less expressed neuropsychiatric symptoms than those treated with placebo, but the results of the study show that these drugs are not effective enough. When a combination of these drugs was administered to the animals with partially ligated CCAs, the symptoms of a psychoneurological deficit, indicators of motor and orienting-exploratory activities, fine motor skills and a cognitive dysfunction were less pronounced, while the differences with the indicators of the animals that had been injected with placebo were statistically significant (p <0.05). That makes it possible to judge on the presence of cerebroprotective properties of the studied combination (Table 1).

The animals that had been modeled for an acute brain injury resulting from the TBI or a HS showed pronounced disorders that manifested themselves as symptoms of a neurological deficit according to the Combs&D'Alecy and Garcia scales, decreased motor and orienting activities, and impaired fine motor skills of the forelimbs. (Table 2). In these animals, a higher water content in the brain tissue was also recorded. In the animals that had been injected with sitagliptin or aminoguanidine after modeling the TBI or a HS, the indicators of a neuropsychiatric deficit did not significantly differ from those recorded in the placebo group. With the administration of these drugs combination, the symptoms of a psychoneurological deficit, as well as the brain edema degree, were significantly lower compared to the group without any treatment in conditions of both pathologies. The results obtained indicate a significant protective effect of the sitagliptin and aminoguanidine combination in the animals with an acute brain injury (ACCI and TBI).

DISCUSSION

Given the disappointing dynamics of the spread of carbohydrate metabolism disorders and obesity, the burden of DM and, accordingly, its complications will increase. Despite significant advances in pharmacology (development of new classes of antihyperglycemic drugs – sodium glucose cotransporter type 2 (SGLT-2) inhibitors, GLP-1 and GLP-1/GIP agonists), the therapy (development of new algorithms and tactics of patients with diabetes' treatment) and a diagnosis (an early detection, an available screening), the effectiveness of the diabetes treatment and the prevention of its complications remain insufficient.

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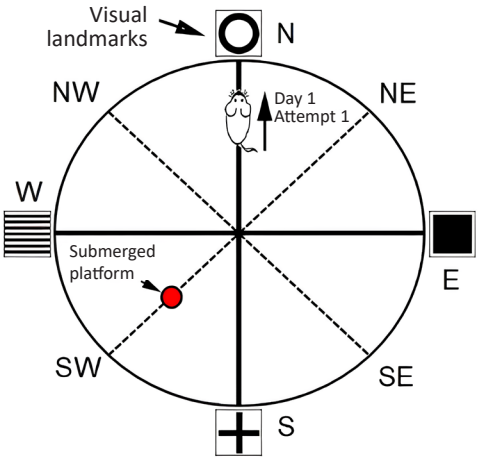

Plan of training

Day	Attempt			
	1	2	3	4
1	N	E	SE	NW
2	SE	N	NW	E
3	NW	SE	E	N
4	E	NW	N	SE

Figure 1 – Layout of installation and scheme of landing sites for animals during training in Morris Water Maze

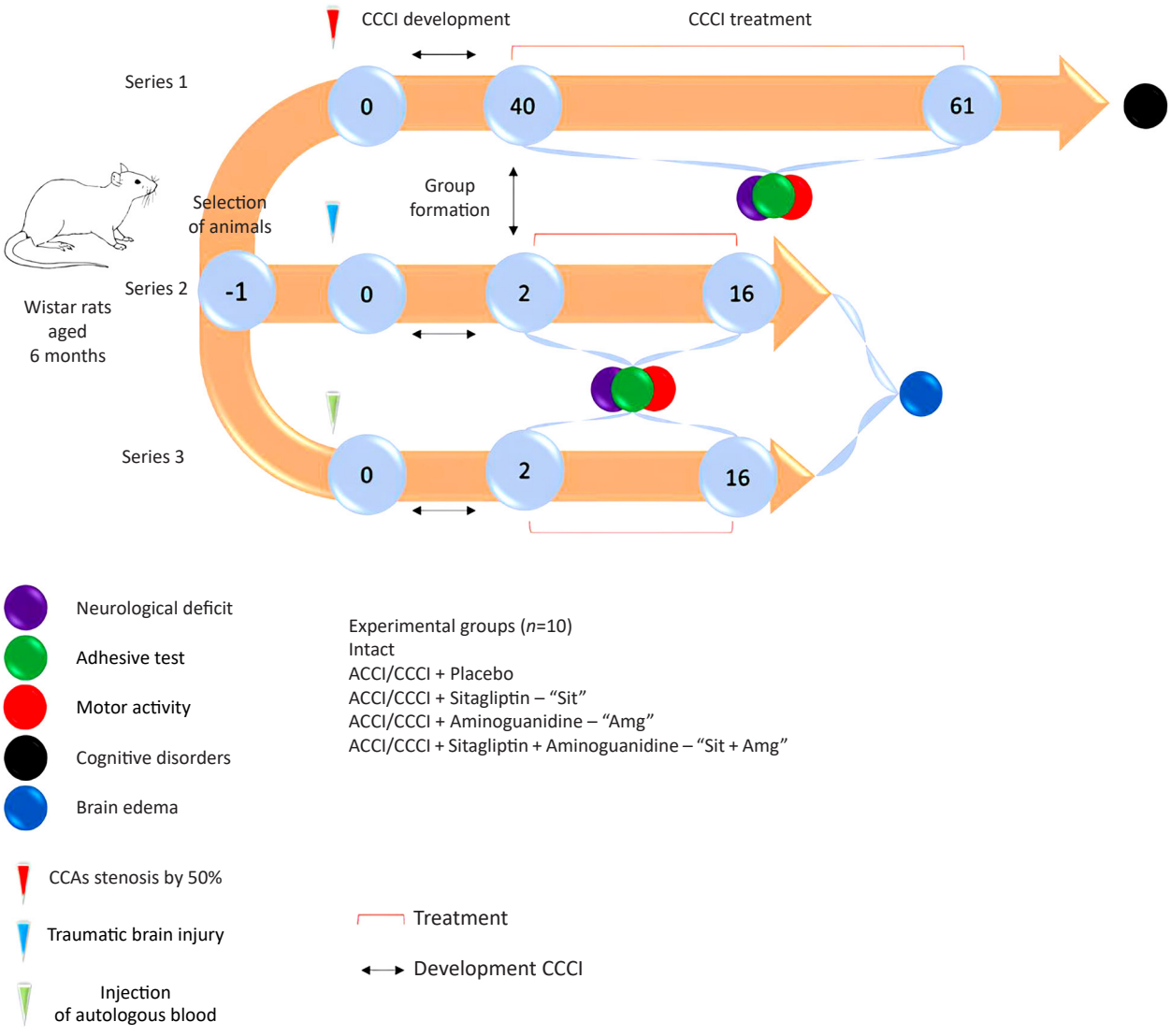

Figure 2 – Study design

Note: CCAs – common carotid arteries, CCCI – chronic cerebral circulation insufficiency, ACCI – acute cerebral circulation insufficiency, Sit – sitagliptin, Amg – aminoguanidine.

Table 1 – Indicators of psychoneurological deficit in CCCI animals

Indicator		Intact	CCCI+			
			Placebo	Sit	Amg	Sit+Amg
mNSS scale, points		0.1±0.1*	4.2±2.1	1.2±0.4*	1.1±1.2	0.5±0.8*
Open field	MA	49.8±15.5*	18.6±7.1	27.3±8	25.6±5.4	35.8±7.8*
	OEA	7.5±1.6*	3.1±0.5	3.4±1.3	3.9±1.6	3.2±1.2
Adhesive test	Average sticker detection time, sec	10.9±5.8*	101.2±41.6	41.7±17.6*	48.4±31.6	33.6±15.8*
	Average sticker removal time, sec	11.2±6.3*	114.2±49.4	43.3±15.7*	53.8±32.2	39.1±11.9*
Morris Water Maze	Day 1, search time, sec	72.3±5.6*	95.8±15.1	89.4±10.7	82.8±5.7	86.8±9.5
	Day 2, search time, sec	59.1±8.2	69.7±19.3	79.4±11.2	69.7±9.5	59.9±7.5
	Day 3, search time, sec	56.3±12.3	57.1±11.1	69.9±10.7	52.1±8.5	45.1±7.9*
	Day 4, search time, sec	32.3±7.3*	51.8±14.3	49.4±10.8	49.8±4.9	37.1±5.2*

Note: MA – motor activity, OEA – orienting-exploratory activity, CCCI – chronic cerebral circulation insufficiency, Amg – aminoguanidine, Sith – sitagliptin, * – differences with the placebo group are significant at p <0.05.

Table 2 – Indicators of psychoneurological deficit in animals with acute brain injury (ACCI and TBI)

Group	Neurological deficit, points		Open field		Adhesive test				Water content in affected hemisphere, %
					Left paw ipsilateral to lesion		Right paw Contralateral to lesion		
	Combs & D'Alecy Scale	Garcia Scale	MA	OEA	Sticker detection time, sec	Sticker removal time, sec	Sticker detection time, sec	Sticker removal time, sec	
Intact	9±0.0*	18±0.0*	42.4±8.2*	25.4±1.6*	15.8±1.1*	23.9±1.4*	14.8±1.3*	19.2±1.2*	76.32±0.03*
TBI+placebo	3±0.1	8.7±0.4	5.1±0.9	5.2±0.3	123.6±15.3	135.9±14.5	180.0±0.0	180.0±0.0	78.58±0.15
TBI+Sit	3.1±0.2	8.7±0.3	4.0±1.2	1.9±0.4	91.1±14.1*	102.1±12.7*	180.0±0.0	180.0±0.0	78.88±0.21
TBI+AMG	3.2±0.2	8.8±0.4	5.2±1.3	1.2±0.2	99.6±12.4	108.9±11.5	180.0±0.0	180.0±0.0	78.19±0.13*
TBI+Sit+ Amg	4.9±0.2*	9.9±0.5	20.5±2.9*	10.5±0.3*	92.9±14.4*	102.7±22.2	180.0±0.0	180.0±0.0	77.83±0.12*
Intact	9±0.0*	18±0.0*	45.2±1.8*	19.4±1.8*	13.9±1.4*	15.8±1.1*	14.8±1.8*	19.2±1.9*	76.06±0.13*
ACCI+ placebo	3±0.3	8.5±0.6	5.5±0.9	3.8±0.4	91.2±11.3	96.8±12.1	180.0±0.0	180±0.0	79.12±0.15
ACCI+Sit	3.9±0.2	9.6±0.3	4.8±0.6	3.2±0.6	80.8±12	83.4±18.2	128.2±10.1*	180±0.0	78.59±0.19*
ACCI+Amg	3.0±0.4	8.7±0.6	5.8±0.3	2.2±0.5	81.8±11	85.9±14.7	135.3±8.1*	180±0.0	78.49±0.29*
ACCI+Sit+ Amg	4.8±0.2*	10.9±0.5*	25.0±1.7*	15.1±2.3*	52.5±11.6*	63.6±11.4*	66±11.8*	75.2±8.1*	78.05±0.24*

Note: TBI – traumatic brain injury, t – time, MA – motor activity, OEA – orienting-exploratory activity, ACCI – acute cerebral circulation insufficiency, Amg – aminoguanidine, Sit – sitagliptin, * – differences with the placebo group are significant at p <0.05.

It is obvious that a compensation of carbohydrate metabolism disorders is the key to the effective prevention of DM complications, but for various reasons, only a small proportion of patients with this diagnosis can reach and maintain the glycemic values set by the doctor. The low availability of modern highly effective antihyperglycemic drugs, as well as the presence of side effects that limit patient adherence to the treatment, are significant obstacles to attempts to manage DM.

The main cause of death in DM remains its cardiovascular complications, the leading cause of which is an endothelial dysfunction (ED) formed under the influence of chronic hyperglycemia and, as a consequence, microcirculation disorders. Even an accidental TBI in DM is much severer, with a higher risk of disability or death [6]. A chronic inflammation and oxidative stress do not only damage the endothelium, exacerbating its dysfunction, but also increase an iNOS expression, which plays a significant negative role under these conditions.

The production of nitric oxide by inducible synthase (iNOS) significantly exceeds that of other isoforms. In addition to the participation of NO in the process of the synthesis and secretion of insulin, microcirculation,

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this molecule is involved in maintaining homeostasis of all body tissues. High concentrations of NO, arising from the activation of iNOS, have a negative effect on the cardiovascular system, increasing an ED. The iNOS activity suppression may play a positive role in relation to the ED and functioning of the insular apparatus [10].

Over the years, various approaches to modulate the iNOS activity – blocking the production of ROS in the upstream direction, the introduction of BH4, the introduction of folic acid for recycling BH2 to BH4, the use of arginase inhibitors, resveratrol, calcium dobesilate, cavnoxin, NOS transcription enhancers (AVE3085 and AVE9488), L-arginine, blockers and activators of various NOS – have been developed [10], but none of them is widely used in medical practice.

Aminoguanidine was developed as a drug for the treatment of diabetes and its complications (nephropathy). This drug combines several potentially valuable properties, i. e., the ability to reduce the iNOS activity and reduce the accumulations of advanced glycation end products. Aminoguanidine was withdrawn from the promotion on the pharmaceutical market for reasons that can be currently revised from the standpoint of modern requirements (changes in indications, additional studies) and opportunities (a molecule optimization, additional studies according to protocols using modern methods of collecting and processing information). Aminoguanidine is also interesting from the point of view of the results of preclinical studies of its cerebroprotective properties in various disorders of cerebral circulation, which is of a high value considering the causes and consequences of diabetes complications.

DPP-4 inhibitors are the first representatives of the class of antihyperglycemic drugs, the mechanism of action of which is associated with the effect on the incretin system, which is involved not only in the regulation of the carbohydrate metabolism, but has also a number of unrelated effects. So, for incretin mimetics to a greater (GLP-1 agonists) or less (iDPP-4) degree, cerebro-, cardio- and endothelioprotective properties have been established [24].

A creation of combined drugs, along with the development of an original drug or its analogue, a new dosage form, a new indication for use, is one of the winning strategies for obtaining a new pharmaceutical product.

Taking into account a wide range of pleiotropic iDPP-4 effects, the list of which is expanding with the accumulation of experience in clinical use, to evaluate a cerebroprotective effect of the sitagliptin and aminoguanidine combination was considered appropriate.

In this study, cerebroprotective effects of the sitagliptin and aminoguanidine combination were established in models of an acute and chronic CCI, and it was found that the protective effect of the combination significantly exceeds the effects of the drugs administered separately, which indicates synergism due to different exposure targets and, accordingly, effects.

CONCLUSION

A combined use of sitagliptin and aminoguanidine has a cerebroprotective effect in a CCI, reproduced by stenosis of the CCAs by 50% and an acute brain injury, reproduced by the application of the TBI or an experimental HS, reducing the severity of neuropsychiatric deficit symptoms, improving cognitive functions and reducing the severity of cerebral brain edema. The sitagliptin and aminoguanidine combination can become the basis for the development of a new promising approach to the treatment of DM 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 – idea and study planning, graphic material design, approval of the 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, Anna V. Kasparova, Sarkis S. Polodyants – statistical data processing, graphic material design, text editing; Dmitry A. Bakulin, Yulia V. Gorbunova, Alexandra V. Baskova – collection and analysis of literature data, manuscript writing. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

REFERENCES

1. Soloveva EYu, Amelina IP. Cerebral small vessel disease's impact on the development of chronic cerebral ischemia: paradigms of treatment. Medical Council. 2020;(2):16–24. DOI: 10.21518/2079-701X-2020-2-16-24. Russian
2. Machinskiy PA, Plotnikova NA, Ul'yankin VE, Kemaykin SP, Rybakov AG. Comparative characteristics of the indicators of mortality and letality from ischemic and hemorrhagic insult in Russia. University proceedings. Volga region. Medical sciences. 2019;3(51):101–18. DOI: 10.21685/2072-3032-2019-3-10. Russian
3. Feigin VL, Krishnamurthi RV, Parmar P, Norrving B, Mensah GA, Bennett DA, Barker-Collo S, Moran AE, Sacco RL, Truelsen T, Davis S, Pandian JD, Naghavi M, Forouzanfar MH, Nguyen G, Johnson CO, Vos T, Meretoja A, Murray CJ, Roth GA; GBD 2013 Writing Group; GBD 2013 Stroke Panel Experts Group. Update on the Global Burden of Ischemic and Hemorrhagic Stroke in 1990-2013: The GBD 2013 Study. Neuroepidemiology. 2015;45(3):161–76. DOI: 10.1159/000441085
4. Voronina VP, Zagrebelnyi AV, Lukina YuV, Kutishenko NP, Dmitrieva NA, Lerman OV, Lukyanov MM, Okshina EYu, Blagodatskikh SV, Ivanova LP, Boytsov SA, Drapkina OM, Martsevich SYu. Features of cerebral stroke course in patients with diabetes mellitus according to the REGION-M register. Cardiovascular Therapy and Prevention. 2019;18(5):60–5. DOI: 10.15829/1728-8800-2019-5-60-65. Russian
5. Galgano M, Toshkezi G, Qiu X, Russell T, Chin L, Zhao LR. Traumatic Brain Injury: Current Treatment Strategies and Future Endeavors. Cell Transplant. 2017 Jul;26(7):1118–30. DOI: 10.1177/0963689717714102
6. Lustenberger T, Talving P, Lam L, Inaba K, Bass M, Plurad D, Demetriades D. Effect of diabetes mellitus on outcome in patients with traumatic brain injury: a national trauma databank analysis. Brain Inj. 2013;27(3):281–5. DOI: 10.3109/02699052.2012.743178
7. Ametov AS, Nevolnikova AO, Tertychnaya EA. The potential of GLP-1 receptor agonists in reducing cardiovascular risk in patients with type 2 diabetes mellitus: what's new? Endocrinology: News, Opinions, Training. 2019;8(3):44–53. DOI: 10.24411/2304-9529-2019-13005. Russian
8. Tyurenkov IN, Bakulin DA, Kurkin DV, Volotova EV. Neuroprotective properties of incretin mimetics in brain ischemia and neurodegenerative diseases. Problems of Endocrinology. 2017;63(1):58–67. DOI: 10.14341/probl201763158-67
9. Kurkin DV, Bakulin DA, Morkovin EI, Strygin AV, Gorbunova YuV, Volotova EV, Makarenko IE, Saparova VB, Drai RV, Petrov VI. Physiology, pharmacology and prospects for dipeptidilpeptidase-4 inhibitors use. Pharmacy & Pharmacology. 2023;11(1):19–47. DOI: 10.19163/2307-9266-2023-11-1-19-47
10. Kurkin DV, Abrosimova EE, Bakulin DA, Kovalev NS, Dubrovina MA, Borisov AV, Strygin AV, Morkovin EI, Tyurenkov IN. Activity modulation of various nitric oxide syntases as an approach to endothelial dysfunction therapy. Pharmacy & Pharmacology. 2022;10(2):130–53. DOI: 10.19163/2307-9266-2022-10-2-130-153
11. Vakili A, Zahedi-Khorasani M. Effect of aminoguanidine on post-ischemic damage in rodent model of stroke. Pak J Pharm Sci. 2008 Jan;21(1):24-8.
12. Vakili A, Hosseinzadeh F, Sadogh T. Effect of aminoguanidine on post-ischemic brain edema in transient model of focal cerebral ischemia. Brain Res. 2007 Sep 19;1170:97–102. DOI: 10.1016/j.brainres.2007.07.016
13. Pérez-Asensio FJ, Hurtado O, Burguete MC, Moro MA, Salom JB, Lizasoain I, Torregrosa G, Leza JC, Alborch E, Castillo J, Knowles RG, Lorenzo P. Inhibition of iNOS activity by 1400W decreases glutamate release and ameliorates stroke outcome after experimental ischemia. Neurobiol Dis. 2005 Mar;18(2):375–84. DOI: 10.1016/j.nbd.2004.10.018
14. Sugimoto K, Iadecola C. Effects of aminoguanidine on cerebral ischemia in mice: comparison between mice with and without inducible nitric oxide synthase gene. Neurosci Lett. 2002 Oct 4;331(1):25–8. DOI: 10.1016/s0304-3940(02)00834-0
15. Thornalley PJ. Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. Arch Biochem Biophys. 2003 Nov 1;419(1):31–40. DOI: 10.1016/j.abb.2003.08.013
16. Kurkin DV, Morkovin EI, Verkholyak DV, Bakulin DA, Volotova EV, Tyurenkov IN. Changes in cerebral blood flow in rats with experimental stenosis of common carotid arteries. Journal of Volgograd State Medical University. 2017;1(61):36–9. Russian
17. Wu G, Bao X, Xi G, Keep RF, Thompson BG, Hua Y. Brain injury after intracerebral hemorrhage in spontaneously hypertensive rats. J Neurosurg. 2011 Jun;114(6):1805–11. DOI: 10.3171/2011.1.JNS101530
18. Kurkin DV, Bakulin DA, Morkovin EI, Kalatanova AV, Makarenko IE, Dorotenko AR, Kovalev NS, Dubrovina MA, Verkholyak DV, Abrosimova EE, Smirnov AV, Shmidt MV, Tyurenkov IN. Neuroprotective action of Cortexin, Cerebrolysin and Actovegin in acute or chronic brain ischemia in rats. PLoS One. 2021 Jul 14;16(7):e0254493. DOI: 10.1371/journal.pone.0254493
19. Dayneko AS, Shmonin AA, Shumeeva AV, Kovalenko EA, Melnikova EV, Vlasov TD. Assessment of neurological deficit in the early and late phase after a 30-minute of focal cerebral ischemia in rats. Regional blood circulation and microcirculation. 2014;13(1):68–78. DOI: 10.24884/1682-6655-2014-13-1-68-78. Russian
20. Bouet V, Boulouard M, Toutain J, Divoux D, Bernaudin M, Schumann-Bard P, Freret T. The adhesive removal test: a sensitive method to assess sensorimotor deficits in mice. Nat Protoc. 2009;4(10):1560–4. DOI: 10.1038/nprot.2009.125
21. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006;1(2):848-58. DOI: 10.1038/nprot.2006.116
22. Han W, Li Y, Cheng J, Zhang J, Chen D, Fang M, Xiang G, Wu Y, Zhang H, Xu K, Wang H, Xie L, Xiao J. Sitagliptin improves functional recovery via GLP-1R-induced anti-apoptosis and facilitation of axonal regeneration after spinal cord injury. J Cell Mol Med. 2020 Aug;24(15):8687–702. DOI: 10.1111/jcmm.15501
23. Sugimoto K, Yagihashi S. Effects of aminoguanidine on structural alterations of microvessels in peripheral nerve of streptozotocin diabetic rats. Microvasc Res. 1997 Mar;53(2):105–12. DOI: 10.1006/mvre.1996.2002
24. Ametov AS, Kamynina LL, Akhmedova ZG. Cardioprotective Effects of Glucagon-Like Peptide 1 Receptor Agonists. Kardiologiia 2014;54(7):92–6. DOI: 10.18565/cardio.2014.7.92-96

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