



# A new EPOR/CD131 heteroreceptor agonist EP-11-1: a neuroprotective effect in experimental traumatic brain injury

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## Abstract

**Introduction:** EP-11-1 (UEHLERLNSS) is a short-chain erythropoietin derivative without have erythropoietic activity. It was created by modifying a peptide mimicking the spatial structure of the erythropoietin a-helix B pHBSF. One of the promising directions of its administration is the correction of morphofunctional disorders that occur in traumatic brain injury (TBI).

**Materials and methods:** The study was performed in 160 male Wistar rats, weighing 180–200 g. TBI was simulated using the drop-weight method. To assess the emerging morphofunctional disorders and a degree of their correction, we used the severity of neurological deficit, indicators of locomotor activity and exploration, a marker of brain injury S100B and morphological examination.

**Results and discussion:** The combined administration of a new EPOR/CD131 heteroreceptor agonist EP-11-1 with [citicoline](#) and [trimetazidine](#) led to a more pronounced correction of the neurological deficit when compared not only to the group of the "untreated" animals, but also to the groups of animals to which these drugs had been administered as monotherapy ( $p < 0.05$ ). The same tendency was also observed in the study of locomotor activity and exploration. A biochemical study showed that the administration of all three combinations led to a statistically significant ( $p < 0.05$ ) decrease in the S-100B concentration compared not only to the group of "untreated" animals, but also to the groups of animals to which these drugs had been administered as a monotherapy.

**Conclusion:** The results of the conducted experiments prove the most pronounced positive dynamics in the combined administration of the new EPOR/CD131 heteroreceptor agonist EP-11-1 with [citicoline](#) and [trimetazidine](#).

## Keywords

traumatic brain injury, secondary failure, [trimetazidine](#), erythropoietin derivatives.

## Introduction

Traumatic brain injury (TBI) is one of the most important problems of public healthcare and public at large. This is due to the prevalence of this type of traumatic injury, the severity of the consequences of TBI, and the significant economic loss caused by TBI (Chen et al. 2018; Jha et al. 2020). At the same time, TBI remains a serious clinical problem worldwide, which affects millions of people every year. There are still not enough medications for the treatment of TBI approved by the FDA (Hubbard et al. 2021).

Brain injury is divided into primary and secondary. The main causes of secondary injury include: increasing edema, extension of hematoma, secondary thrombosis, etc., which lead to the development of energy deficiency, excitotoxicity, oxidative stress, lipid peroxidation, neuroinflammation, inhibitory neurotransmitters deficiency, and apoptosis of the brain tissue cells (Chodobski et al. 2011; Zhang et al. 2012; Jha et al. 2020; Hubbard et al. 2021). It is obvious that in the presence of several factors triggering the various mechanisms of metabolic disorders leading to the secondary injury, a complex therapy is the only correct one. The latter should include a combination of medications complementing one another in the mode of action and influence on various pathogenetic processes.

In this regard, a promising point for the correction of morphofunctional disorders in TBI may be the prevention of an increase in  $Ca^{2+}$  within a cell and mitochondria, which will lead to a decrease in energy deprivation, inhibition of the mitochondrial macropore opening, and activation of caspases and apoptosis. In addition, a suppression of the glutamate release to some extent will reduce the phenomenon of excitotoxicity (Morin et al. 2000).

Another way to reduce excitotoxicity can be through the modulation of the release/absorption processes for such neurotransmitters as glutamate and GABA by platelets (Leiter and Walker 2020). It is also important that the prevention of platelet activation will lead to a decrease in the secretion of chemokines, and, consequently, the migration of immune competent cells to the affected area. With a decrease in the release of TGF- $\beta$  by the immune competent cells, this will lead to a decrease in neuroinflammation (Chodobski et al. 2011; Gianazza et al. 2020; Hubbard et al. 2021). In addition, the antiplatelet action is one of the ways of secondary thrombosis prevention.

There is some evidence of good prospects of using EPOR/CD131 heteroreceptor agonists. At the same time, various cytoprotective effects are realized by the activation of multiple paths: anti-ischemic, anti-apoptotic, anti-oxidant, endothelioprotective, etc. (Brines et al. 2015; Xiong et al. 2015; Golubev et al. 2020a; Lokteva et al. 2020).

Another promising mechanism to be logically included in the complex therapy is the phospholipase A2 inhibition. This will slow down the membrane phospholipid degradation and the formation of arachidonic acid. On the one hand, this will manifest in a membrane-stabilizing effect, and, on the other hand, in a decrease in neuroinflammation and the formation of reactive oxygen species (Jasielski et al. 2020).

The classic activator of the EPOR/CD 131 heteroreceptor is **erythropoietin**, which has an erythropoietic activity and a large size as disadvantages. **Erythropoietin** short-chain derivatives, the classic example of which is pHBPS, are most promising in this regard (Brines et al. 2015; Xiong et al. 2015; Korokin et al. 2020a). The possibility to give them additional properties by modifying the polypeptide chain, in particular, the antiplatelet activity with the cytoprotective activity remaining at the same level, is of major importance (Golubev et al. 2020b; Gureev et al. 2020; Korokin et al. 2020b; Puchenkova et al. 2020). For the study, we selected a polypeptide EP-11-1 (UEHLERALNSS), which has antiplatelet and cytoprotective activities (Antsiferov et al. 2020; Korokin et al. 2020c; Korokin et al. 2021).

In connection with the above, when selecting the combinations of pharmacological agents for the study, the choice was made in favor of **citicoline** because of its ability to inhibit phospholipase A2, to modulate the release of various neurotransmitters, promoting neuroregeneration, and to inhibit apoptosis through the mitochondrial mechanisms (Iulia et al. 2017; Gandolfi et al. 2020; Jasielski et al. 2020). Another medication to be logically included in the complex therapy is **trimetazidine**, which has anti-excitotoxic, anti-apoptotic and anti-ischemic effects. Literature data available suggest the neuroprotective activity of **trimetazidine**, which indicates its ability to pass through the blood-brain barrier (Morin et al. 2000; Demir et al. 2010; Al-Kuraishy et al. 2017).

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**Aim of the study:** to demonstrate the prospects of administering a new EPOR/CD131 heteroreceptor agonist – EP-11-1 – for the correction of the morphofunctional disorders in experimental TBI in rats.

## Materials and methods

The experiment was performed at the Center for Preclinical and Clinical Studies of Belgorod State National Research University. “The Rules of Laboratory Practice”, approved by Order No.708n of the Ministry of Health of the Russian Federation of 23.08.2010, in strict compliance with “The European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes” (Directive2010/63/EU). The experimental studies were approved by the Bioethical Commission of Belgorod State National Research University. Vivisection was performed in compliance with the ethical principles of treating laboratory animals outlines in “The European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes”. CETS No.123.

### Experimental animals

The experimental study was performed in 160 male Wistar rats, weighing 180–200 g. The animals were kept in accordance with the rules of laboratory practice for preclinical

studies on the territory of the Russian Federation. The animals were kept under the standard conditions corresponding to the sanitary rules on the organization, equipment and maintenance of experimental biological clinics (vivariums) No. 1045-73, approved by the Ministry of Health of the USSR on 06.04.1973 and GOST R 53434-2009. The individually ventilated cages (Tecniplast S.p.A., Italy) designed for keeping small laboratory animals. The bedding was sawdust, sterilized by ultraviolet irradiation. Special pellet feed for small laboratory rodents and pre-treated water disinfected with UV irradiation were used. In each cage, microclimate was created and supported by an individual ventilation system. All the animals had been acclimatized and quarantined for at least 10 days before the experiment.

### Simulation of traumatic brain injury in laboratory animals

Drop-weight TBI model was used in this study (Martynova et al. 2019; Cherevatenko et al. 2020).

### Assessment of neurological deficit

To assess the neurological deficit of the laboratory animals and its dynamics during the study, the following evaluation scales were used: the Stroke-Index McGraw scale modified by I. V. Gannushkina (Martynova et al. 2019; Plotnikov et al. 2019), Combs and D'Alecy scale (Doepfner et al. 2014; Kurkin et al. 2021), and Garcia scale (Desland et al. 2014; Martynova et al. 2016).

### Methods for assessing the behavioral status of the laboratory animals

The impact of TBI and the studied compounds on behavior was evaluated in a standard Open Field behavioral test (Martynova et al. 2019; Agarkova et al. 2021). During the test, the Smart 3.0 video tracking system software (Panlab Harward Apparatus) was used, a modified Hall's test ("open field") with automated video monitoring. This software makes it possible to automatically (in real time) analyze such indicators of animals' activity as their average speed, the distance covered, time spent in pre-defined zones of the "Open Field", and the rearing activity (Jaglin et al. 2018; Martynova et al. 2019).

### Assay of S100B brain damage marker

As a component of assessing the severity and dynamics of the condition of the animals with experimental TBI, we evaluated the level of S100B protein in the animals' blood. Blood was sampled in all the studied groups on day 7. To do this, the left ventricle was punctured intercostally in the projection of the heart, and 2 ml of blood was sampled into the vacutainer. The concentration of S100B in blood was determined by the electrochemiluminescence immunoassay (ECLIA), using the test system for quantitation in vitro S100 (S100 A1B and S100 BB).

### Morphometric technique for the study of animals' brain tissues

In the studied groups, after TBI simulation (intact group after anesthesia), in compliance with the principles of humane treatment of animals, the animals were sacrificed under anesthesia (chloral hydrate, 350 mg/kg) by a left ventricular puncture until complete bleeding.

For a morphological examination, cephalotomy was performed, followed by the extraction of the brain with the cerebellum. The resulting biomaterial (brain) was fixed in a 10% solution of neutral formalin. After fixation, a part of the cerebral cortex in the area of injury (frontoparietal region) was sectioned and embedded into paraffin, according to the standard method. Next, the sections, 5–7 microns thick, were made and stained with hematoxylin and eosin. Microscopy and photographing were carried out using an optical system consisting of a Leica CME microscope and a DCM-510 eyepiece camera at magnifications of  $\times 100$ -,  $\times 200$ - and  $\times 400$ -fold, with documenting the images in the FUTURE WINJOE software included in the eyepiece camera package.

### Dose selection rationale

During the experimental study, the neuroprotective effect of trimetazidine was studied. Citicoline was used as a comparison drug. The possible results of the combined neuroprotective therapy were also evaluated.

Trimetazidine (Preductal MR, Servier, Russia) was administered 2 hours before the TBI simulation, intragastrically, at a dose of 6 mg/kg/day; the dose was divided into two administrations, with an interval of 12 hours, and the therapy was carried out throughout the entire period of the experiment (7 days) or before the animal was removed from the experiment. This dose corresponds to the recommended therapeutic dose in humans of 1 mg/kg (70 mg/day); the dose of the medication was recalculated using the generally accepted formula for interspecific dose scaling according to (Freireich M. et al. and Ulanova I. P).

Citicoline (Ceraxon, Ferrer Internacional, S. A., Spain): at an acute ischemic stroke and TBI, it is recommended to use the following dose schedule: 1000 mg every 12 hours from the first day after the diagnosis. Based on this, the dose for a rat weighing 180–200 g was calculated using the generally accepted formula for interspecific dose scaling. The drug administration was started 2 hours before the TBI simulation, intraperitoneally, at a dose of 170 mg/kg/day; the dose was divided into two administrations with a 12-hour interval, at the same point of time; the therapy was performed throughout the entire period of the experiment (7 days) or before the animal was removed from the experiment.

A new EPOR/CD131 heteroreceptor agonist EP-11-1 (UEHLERLNSS): the drug was administered 2 hours before the TBI simulation, intraperitoneally, at a dose of 20 mcg/kg/day (Golubev et al. 2020a, 2020b).

All the experimental animals were divided into the following groups:

- 1 group – intact animals;
- 2 group – TBI;
- 3 group – EP-11-1 (20 mcg/kg/day);
- 4 group – TBI + **Citicoline** (170 mg/kg/day);
- 5 group – TBI + **Trimetazidine** (6 mg/kg/day);
- 6 group – EP-11-1 (20 mcg/kg/day) + **Citicoline** (170 mg/kg/day)
- 7 group – EP-11-1 (20 mcg/kg/day) + **Trimetazidine** (6 mg/kg/day)
- 8 group – **Trimetazidine** (6 mg/kg/day) + **Citicoline** (170 mg/kg/day)

### Statistical data processing

The statistical data were processed using the Statistica 10.0 software. Shapiro-Wilk and Spiegelhalter (normtest package) normality tests were performed for the obtained data; the equality of variances was assessed using the Levene's test (lawstat package). Depending on the type of distribution and the equality of variances, the significance of the results obtained was evaluated using parametric (ANOVA) or non-parametric (Kruskal-Wallis test) one-way analysis of variance, and as a post-hoc analysis to identify intergroup differences, the Student's t-test or the Mann-Whitney test were used, respectively, with the Benjamini-Hochberg correction for multiple tests. The results were considered reliable at  $p \leq 0.05$ .

## Results and discussion

The TBI simulation led to a severe neurological deficit in the animals with some regression on day 7. In the groups administered with these pharmacological agents as a monotherapy, the indicators were statistically significantly different from the group of "untreated" animals (TBI) ( $p < 0.05$ ) (Table 1). However, the level of the intact group of animals was not reached.

The combined administration of a new EPOR/CD131 heteroreceptor agonist EP-11-1 with **citicoline** and **trimetazidine** led to a more pronounced improvement

compared not only to the group of "untreated" animals, but also to the groups of animals administered with these drugs as a monotherapy ( $p < 0.05$ ) (Table 1). But the target level was not reached. In the group of animals administered with a combination of **trimetazidine** and **citicoline**, a statistically significant decrease in neurological deficit was observed compared to the TBI group, but there was no significant difference compared to the groups administered with these drugs as a monotherapy.

The TBI simulation led to a pronounced decrease in locomotor activity and exploration, as evidenced by a statistically significant ( $p < 0.05$ ) decrease in the number of free-rearings and wall-rearings, and the distance covered for 3 minutes, as well as the time spent in the central zones compared to the group of the intact animals (Table 1). By day 7, there was some improvement in these indicators, but their level was significantly lower than in the intact animals ( $p < 0.05$ ).

Monotherapies with the studied pharmacological agents improved the indicators of the locomotor activity and exploration on day 7; after the TBI simulation, as evidenced by a statistically significant ( $p < 0.05$ ) increase in the number of free-rearings and wall-rearings, and an increase in the distance covered for 3 minutes compared to the group of the "untreated" animals. In addition, in these groups, there was a statistically significant increase in the time spent in the central zones ( $p < 0.05$ ) (Fig. 1), but the level of the intact animals was not reached.

A study of locomotor activity and exploration on day 7 showed that a combined administration of EP-11-1 with **citicoline** statistically significantly increased the number of free-rearings and wall-rearings in comparison with the groups administered with these pharmacological agents as a monotherapy. In the group of animals administered with a combination of EP-11-1 with **citicoline** and **trimetazidine**, the distance covered reached the level of the intact animals, and the combined administration of EP-11-1 with **trimetazidine** had a statistically significant advantage in comparison with the same pharmacological agents used as a monotherapy.

In addition, in these groups, there was a statistically significant increase in the time spent in the central zones ( $p < 0.05$ ) (Table 2). In the groups of animals that

**Table 1.** The Effect of the Combined Administration of New EPOR/CD131 Heteroreceptor Agonist EP-11-1 on the Neurological Deficit in TBI

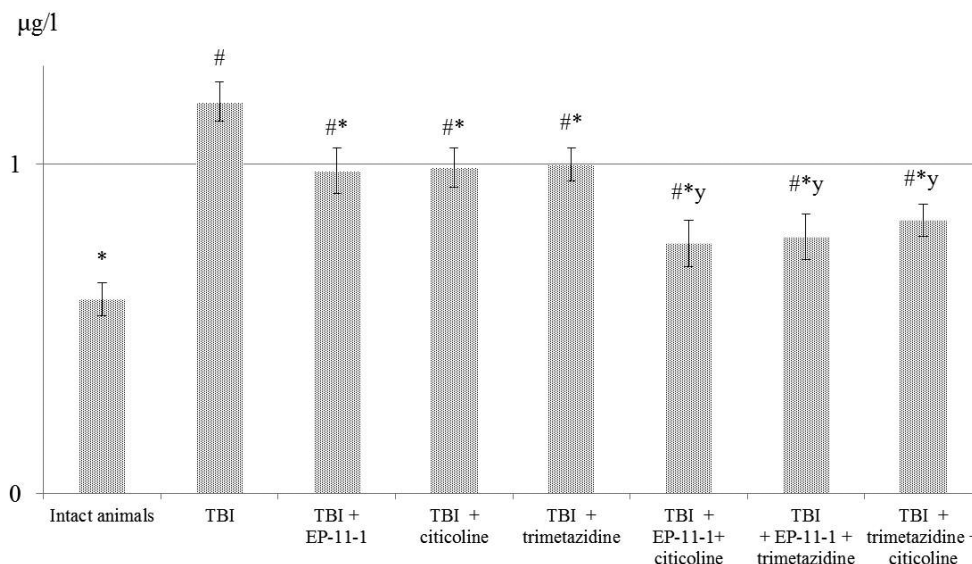
Scale	McGraw modified by I. V. Gannushkina	Garcia	Combs and D'Alecy
Medication			
Intact animals	0*	18*	9*
TBI	2.96 <sup>#</sup>	11.38 <sup>#</sup>	5.85 <sup>#</sup>
TBI + EP-11-1	1.96 <sup>#*</sup>	14.79 <sup>#*</sup>	7.14 <sup>#*</sup>
TBI + <b>citicoline</b>	2.11 <sup>#*</sup>	14.29 <sup>#*</sup>	6.93 <sup>#*</sup>
TBI + <b>trimetazidine</b>	1.96 <sup>#*</sup>	14.38 <sup>#*</sup>	7.15 <sup>#*</sup>
TBI + EP-11-1 + <b>citicoline</b>	1.29 <sup>#*</sup>	15.93 <sup>#*</sup>	8.14 <sup>#*</sup>
TBI + EP-11-1 + <b>trimetazidine</b>	1.18 <sup>#*</sup>	16.07 <sup>#*</sup>	8.21 <sup>#*</sup>
TBI + <b>trimetazidine</b> + <b>citicoline</b>	1.88 <sup>#*</sup>	15.23 <sup>#*</sup>	7.62 <sup>#*</sup>

Note: <sup>#</sup> –  $p < 0.05$  in comparison with the intact animals; \* –  $p < 0.05$  in comparison with the animals with TBI; <sup>γ</sup> –  $p < 0.05$  in comparison with monotherapy.

**Table 2.** The Effect of New EPOR/CD131 Heteroreceptor Agonist EP-11-1 on the Locomotor Activity and Exploration on Day 7 in TBI

Day	Number of free-rearings and wall-rearings	Distance covered (m)	Time spent in the central zone
Medication			
Intact animals	16.2 ± 0.89 <sup>*</sup>	23.47 ± 1.00 <sup>*</sup>	2.94 ± 0.24 <sup>*</sup>
TBI	5.8 ± 0.59 <sup>#</sup>	9.33 ± 0.95 <sup>#</sup>	0.95 ± 0.21 <sup>#</sup>
TBI + EP-11-1	11.0 ± 0.67 <sup>#*</sup>	19.40 ± 0.99 <sup>#*</sup>	2.16 ± 0.20 <sup>#*</sup>
TBI + <b>citicoline</b>	9.8 ± 1.0 <sup>#*</sup>	16.86 ± 1.02 <sup>#*</sup>	2.04 ± 0.15 <sup>#*</sup>
TBI + <b>trimetazidine</b>	10.3 ± 0.93 <sup>#*</sup>	17.77 ± 1.22 <sup>#*</sup>	2.07 ± 0.16 <sup>#*</sup>
TBI + EP-11-1 + <b>citicoline</b>	13.6 ± 0.64 <sup>#*</sup>	21.51 ± 0.82 <sup>*</sup>	2.51 ± 0.23 <sup>*</sup>
TBI + EP-11-1 + <b>trimetazidine</b>	13.0 ± 0.73 <sup>#*</sup>	21.97 ± 0.70 <sup>γ*</sup>	2.79 ± 0.22 <sup>*</sup>
TBI + <b>trimetazidine</b> + <b>citicoline</b>	11.1 ± 0.66 <sup>#*</sup>	19.18 ± 0.95 <sup>#*</sup>	2.21 ± 0.2 <sup>#*</sup>

Note: <sup>#</sup> –  $p < 0.05$  in comparison with the intact animals; \* –  $p < 0.05$  in comparison with the animals with TBI; <sup>γ</sup> –  $p < 0.05$  in comparison with monotherapy.



**Figure 1.** The effect of new EPOR/CD131 heteroreceptor agonist EP-11-1 on the S-100B concentration on day 7 in TBI. **Note:** # –  $p < 0.05$  in comparison with the intact animals; \* –  $p < 0.05$  in comparison with the animals with TBI;  $y$  –  $p < 0.05$  in comparison with monotherapy.

had been administered with the combinations of EP-11-1 with **citicoline** and **trimetazidine**, the time spent in the central zones reached the level of the intact animals ( $p < 0.05$ ).

The study of biochemical markers of brain injury (protein S100B) showed its statistically significant increase ( $p < 0.05$ ) in the blood on day 7 after the TBI simulation compared with the group of intact animals (Fig. 1). The administration of the studied pharmacological agents as a monotherapy to the animals with TBI led to a statistically significant ( $p < 0.05$ ) decrease in the S-100B level in the blood on day 7 after the TBI simulation compared with the group of the “untreated” animals. This suggests less brain damage in these groups, but the target level was not reached.

The study of biochemical markers of brain injury (protein S100B) in blood plasma showed that the administration of the combination of **trimetazidine** and a new EPOR/CD131 heteroreceptor agonist EP-11-1 with **citicoline**, as well as their combinations, led to a statistically significant ( $p < 0.05$ ) decrease in S-100B in the blood on day 7 day after the TBI simulation compared with the group of the “untreated” animals. This suggests less brain damage in these groups. In addition, in the described groups, the effect was statistically significantly ( $p < 0.05$ ) superior to the effects of the same pharmacological agents in the groups with monotherapy (Fig. 1).

A microscopic examination of the histological sections of the cerebral cortex after the TBI simulation showed clear signs of brain damage, which were accompanied by an increase in the cross section size of neurons and a decrease in the density of neurons and gliocytes (Table 3). The administration of the studied pharmacological agents as a monotherapy to the animals with TBI led to a statistically significant ( $p < 0.05$ ) improvement in their morphometric parameters on day 7

**Table 3.** The Effect of New EPOR/CD131 Heteroreceptor Agonist EP-11-1 on the Morphometric Parameters in TBI

Day	Cross section of the neurons (mcm <sup>2</sup> )	Number of neurons in the standard section area	Number of gliocytes in the standard section area
Intact animals	61.05 ± 0.74*	60.80 ± 1.32*	67.85 ± 1.03*
TBI	68.40 ± 0.99#	36.45 ± 1.30#	80.80 ± 1.56#
TBI + EP-11-1	62.70 ± 0.86*	56.55 ± 0.91#*	71.70 ± 1.42#*
TBI + <b>citicoline</b>	62.50 ± 1.17*	45.10 ± 1.26#*	80.00 ± 1.91#*
TBI + <b>trimetazidine</b>	63.95 ± 0.88#*	45.75 ± 1.36#*	73.90 ± 1.36#*
TBI + EP-11-1 + <b>citicoline</b>	59.75 ± 0.70*	59.65 ± 1.12*	69.70 ± 1.44*
TBI + EP-11-1 + <b>trimetazidine</b>	60.75 ± 0.85*	60.60 ± 1.34*	67.20 ± 1.31*
TBI + <b>trimetazidine</b> + <b>citicoline</b>	63.00 ± 1.11*	56.05 ± 0.78 $y$	65.25 ± 1.22 $y$

**Note:** # –  $p < 0.05$  in comparison with the intact animals; \* –  $p < 0.05$  in comparison with the animals with TBI;  $y$  –  $p < 0.05$  in comparison with monotherapy.

day after the TBI simulation compared with the group of the “untreated” animals and a positive dynamics of the visually evaluated histologic pattern. This suggests less brain damage in these groups, but the target level was not reached.

On day 7 in the groups administered with the studied combinations, the morphometric parameters were at the level of the intact animals. In the group with the combined administration of **trimetazidine** and **citicoline**, an increase in the neuron and gliocyte density significantly exceeds that in the groups of animals administered with the same pharmacological agents as a monotherapy ( $p < 0.05$ ).

Thus, the results of the experiments indicate the most pronounced positive dynamics under the influence of the combined administration of **trimetazidine** and a new EPOR/CD131 heteroreceptor agonist EP-11-1 with **citicoline**, as well as their combinations in TBI. This is evidenced by the more pronounced effects of the combinations on the correction of morphofunctional disorders in simulated TBI compared to the effects of the same pharmacological agents used as a monotherapy.

## Conclusion

Primary injury in TBI is accompanied by a number of disorders that trigger a cascade of destructive biochemical processes that lead to secondary injury of brain cells.

The main causes of secondary damage include: increasing edema, hematoma extension, secondary thrombosis, etc. They lead to the development of energy deficiency, excitotoxicity, oxidative stress, lipid peroxidation, neuroinflammation, inhibitory neurotransmitters deficiency, and apoptosis of the brain tissue cells (Chodobski et al. 2011; Zhang et al. 2012; Jha et al. 2020; Hubbard et al. 2021).

Administration of the studied pharmacological agents as a monotherapy led to the correction of morphofunctional disorders occurring in simulated TBI. The pronounced neuroprotective activity of a new EPOR/CD131 heteroreceptor agonist EP-11-1 is partially mediated by EPOR/CD131. Autophosphorylation of Janus 2 (Jak2) kinase activates three main cascades. First, this is a transcription pathway that includes STAT3 and STAT5, which leads to amplified signals of survival and resistance to apoptosis (Brines 2014). The next cascade includes a phosphatidylinositol-3'-kinase (PI3K) and the Akt pathway. The PI3K/Akt pathway phosphorylates a kinase of the glycogen synthase 3 $\beta$  (GSK3 $\beta$ ), significantly reducing its activity, inhibiting the mitochondrial macropore opening and stabilizing the mitochondria, which also leads to the inhibition of apoptosis. The inhibition of GSK3 $\beta$  also suppresses the nuclear factor-kB (NF-kB), thereby reducing the neuroinflammation development. The next pathway is mitogen-activated protein kinase (MAPK), which also inhibits GSK3 $\beta$  and reduces the inflammation (Cruz et al. 2014). In addition, the PI3K/Akt pathway stimulates the NO production by the activation of endothelial nitric oxide synthase (eNOS), which increases blood flow, reduces regional trauma and induces the endothelial cell proliferation, migration and healing (Peng et al. 2020).

Antiplatelet activity of EP-11-1 facilitates the modulation of the processes of release/absorption of such neurotransmitters as glutamate and GABA. At the same time, a decrease in the release of glutamate and the absorption of GABA by platelets occurs, which reduces the excitotoxicity (Leiter and Walker 2020). It is also important that the prevention of platelet activation will lead to a decrease in the secretion of chemokines, and, consequently, the migration of immune competent cells to the affected area. Together with a decrease in TGF- $\beta$  release by these cells, it will lead to a decrease in the neuro-inflammation (Chodobski et al. 2011; Gianazza et al. 2020; Hubbard et al. 2021). In addition, the antiplatelet effect is one of the ways for the prevention of secondary thrombosis.

The positive effects of trimetazidine can be associated with the activation of mitochondrial and cytoplasmic  $\sigma_1$  receptors and the inhibition of 3-ketoacyl-CoA thiolase. This leads to a decrease in the calcium intake to the cell and its mobilization from the intracellular depot. This prevents an increase in Ca<sup>2+</sup> inside the cell, the release of

glutamate, and, in part, prevents the development of excitotoxicity. The prevention of mitochondrial Ca<sup>2+</sup> overload prevents energy starvation, inhibits the mitochondrial macropore opening, activation of caspases and the start of apoptosis (Morin et al. 2000). In this case, inhibition of the mitochondrial macropore opening can be considered as a variant of pharmacological preconditioning in relation to secondary brain injuries in TBI. Another mechanism of action is the exclusion of fatty acids in glial cells from the energy metabolism and its conversion to carbohydrates. This leads to a more efficient ATP formation. In addition, trimetazidine reduces the Bax/Bcl-2 ratio (Zhang et al. 2019), which also slows down the start of apoptosis.

The positive effects of citicoline in the combined administration of the studied pharmacological agents on the correction of morphofunctional disorders in TBI are realized due to its complex neuroprotective effect. Citicoline inhibits phospholipase A2. This mechanism leads to inhibition of the membrane phospholipids cleavage and the formation of arachidonic acid. On the one hand, this increases the stability of biological membranes; on the other hand, it reduces the neuroinflammation and the formation of reactive oxygen species. Ultimately, this helps to reduce apoptosis. There is experimental evidence that the anti-apoptotic effect of citicoline is realized by mitochondrial mechanisms, as well as by the increased expression of anti-apoptotic protein Bcl-2 (Gandolfi et al. 2020; Kamel et al. 2020; Nashine and Kenney 2020). In addition, the ability of citicoline to activate neuroregeneration also contributes to a quicker improvement of morphofunctional disorders in TBI (Gandolfi et al. 2020; Martí-Carvajal et al. 2020).

Thus, in conclusion, it should be noted that the most pronounced positive neuroprotective effect observed for the combined administration of the studied pharmacological agents is explained by their influence on various pathogenic points of the development of secondary injury in TBI.

## Conclusion

1. The administration of a new EPOR/CD131 heteroreceptor agonist EP-11-1, obtained as a result of BLAST screening, for the correction of morphofunctional disorders occurring in simulated TBI in rats has a pronounced neuroprotective effect. This is supported by a reduction in neurological deficit on day 7 and an improvement in the indicators of locomotor activity and exploration, psycho-emotional state, an 18% decrease in the plasma content of the S-100B protein, which is a marker of nerve tissue damage, a decrease in the cross-section of neurons to the level of the intact animals on day 7 and a statistically significant increase in the density of neurons and gliocytes in brain tissue.
2. Trimetazidine has a pronounced neuroprotective effect in the correction of the morphofunctional disorders occurring in simulated TBI, as evidenced by the reduction in neurological deficit on day 7 and an

improvement in locomotor activity and exploration, psychoemotional state, a 16% decrease in the plasma content of the S-100B protein, which is a marker of nerve tissue damage, a decrease in the cross-section of neurons and an increase in the density of neurons and gliocytes in brain tissue.

3. The combined administration of **trimetazidine** and a new EPOR/CD131 heteroreceptor agonist EP-11-1 with **citicoline** leads to a more pronounced correction of morphofunctional disorders in simulated TBI compared to the animal groups treated with these pharmacological agents as a monotherapy. This is evidenced by a statistically significant reduction in neurological deficit on day 7, an increase in the number of free-rearings and wall-rearings in the group with the combined administration of a new EPOR/CD131 heteroreceptor agonist EP-11-1 with **citicoline** compared to the animal groups administered with these pharmacological agents as a monotherapy. The distance covered in the "open field" test and the time spent in the central zones in this group was comparable to those in the group of intact animals. The level of the marker of brain injury – S100B protein – in the groups with the combined administration of the studied pharmacological

agents decreased by 1.4 times and 1.6 times, respectively, and statistically significantly differed from the groups of animals treated with these pharmacological agents as monotherapy, and morphometric indicators reached the level of the intact animals.

4. The combination of **trimetazidine** and a new EPOR/CD131 heteroreceptor agonist EP-11-1 significantly exceeded the effects of the same drugs used as a monotherapy. On day 7, there was a statistically significant improvement in the neurological deficit compared to the groups with a monotherapy. The main indicators of the psychoemotional state on day 7 were comparable with those in the group of intact animals, and the marker of brain injury – S100B protein – statistically significantly decreased compared to the groups of animals treated with these pharmacological agents in a monotherapy. The morphometric indicators in this group were significantly better than in the groups with a monotherapy on day 3, and on day 7, they reached the levels of the intact animals.

## Conflict of interest

The authors declare no conflict of interests.

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