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V. A. Tkachenko[1], A. I. Shevtsova[2], A. E. Lievykh[1], Yu. V. Kharchenko[1], V. I. Zhyliuk[1]

Study of edaravone impact on oxidative stress biomarkers in serum and brain of rats with traumatic brain injury

[1]Dnipro State Medical University, Dnipro
[2]Oles Honchar Dnipro National University, Dnipro

Key words: traumatic brain injury, biomarkers, oxidative stress, edaravone, brain, serum

It is known that traumatic brain injury (TBI) is one of the main causes of death and disability among people of all ages. Recently, the risk of TBI has increased dramatically in Ukraine, not only for the military, but also for civilians. After an acquired TBI a cascade of multidirectional metabolic changes begins in the brain, including protein carbonylation, increased lipid peroxidation, free radicals formation, impaired neurotransmitters release and energy imbalance, which are associated with the development of various functional disorders [1].

The severity of metabolic changes always correlates with the degree of brain damage and various additional factors. In the case of severe traumatic brain injury, we should expect such possible consequences as speech, memory and attention disorders or paralysis. Many researchers note, that learning and memory dysfunction after TBI involve much more complex pathologies, including neuronal death and dysfunction in the synapse, hippocampus, or brain network [2, 3]. Early rehabilitation and the choice of adequate treatment, that would mitigate the destructive effects on the brain and nervous system functioning are essential. Therefore, the search for new effective drugs remains relevant.

In the context of this problem, it is possible to use a strong antioxidant, such as edaravone (Eda), due to its ability to eliminate hydroxyl, peroxide, and superoxide (O_2^-) radicals, which cause damage to neurons and blood vessels [4]. Edaravone reduces reactive oxygen species (ROS) and attenuates inflammatory reactions after cerebral ischemia in animals and humans; it reduces post-ischemic inflammation, which leads to brain swelling and stroke due to damage of neurons and endothelial cells. The use of edaravone to treat many diseases associated with oxidative stress is becoming widespread. More and more studies indicate that this drug has promising potential for the treatment of certain neurological disorders, such as stroke (ischemic, hemorrhagic), Parkinson's disease, spinal cord injury, amyotrophic lateral sclerosis and others [5].

The aim of the study was to examine the effect of edaravone on biomarkers of carbonyl-oxidative stress in rats with traumatic brain injury.

Materials and methods. *Experimental animals.* The study was carried out on 30 male Wistar rats weighing 200–250 g. The study design was approved by the Biomedical Ethics Committee of Dnipro State Medical University (protocol No. 1 dated 21.09.2022). Experiments were performed in compliance with Directive

86/609/EEC on the protection of animals used for experimental and other scientific purposes. The animals were kept in standard vivarium conditions (air temperature 22–24 °C, relative humidity 50%, 12-hour day/night cycle) with free access to water and food.

Modeling of traumatic brain injury. TBI was caused by mechanical damage from a metal weight (450 g), which was free-falling in a vertical pipe from a height of 170 cm onto the rat head [6]. During TBI modeling, rats were under general anesthesia (zolazepam 15 mg/kg and tiletamine 15 mg/kg, intraperitoneally). The rats were placed in individual cages with a bedding temperature of 27–32 °C, covered with a synthetic cloth. The cages were heated until recovery from anesthesia, but not less than 12 hours.

Experimental groups. All animals were randomized into 3 groups: negative control – intact rats (n = 10); positive control – rats with TBI (n = 8); rats with TBI + Eda 6 mg/kg, intraperitoneally (n = 10). Administration of Eda was performed 1 hour, 24 hours and 48 hours after TBI. The animals were sacrificed (under general anesthesia) 1 hour after the last dose of Eda.

Oxidative stress biomarkers. Advanced oxidation protein products (AOPP) were assayed using the modified method of Witko-Sarsat et al. Thiobarbituric acid reactive substances (TBARS) were evaluated by the reaction of lipid peroxides with thiobarbituric acid; the level of carbonylated proteins (PC370/PC430) were evaluated by the reaction of protein carbonyl derivatives (ketone-2,4-dinitro-phenylhydrazone) with 2,4-dinitro-phenylhydrazine (DNPH). Ischemia modified albumin (IMA) was assayed using the albumin cobalt-binding test. For the determination of lactate levels, a standard lactate test kit was used. All oxidative stress biomarkers were studied using spectrophotometry [7–10] in serum and fraction S1 from the brain cortex and hippocampus of experimental animals.

Statistical analysis was performed by GraphPad Prism 9.0 (GraphPad Software, Inc., La Jolla, CA, USA, GPS-2169913-THSG-DF1FF). All quantitative results were presented using box-and-whisker plots, which display the distribution of the data through their median, lower/upper quartiles and smallest/largest values. Data normality was assessed using the Shapiro-Wilk test. Statistical significance ($P < 0.05$) was determined by a two-tailed Student t-test or one-way ANOVA for normally distributed variables; and the Mann-Whitney U-test for non-normally distributed variables.

Results and discussion. As shown in Figure 1, the levels of PC430 and AOPP and lactate in serum of rats with TBI were increased compared to the intact rats by 40% ($P = 0.09$) and 27% ($P < 0.05$), respectively. There were no significant changes in the serum levels of TBARS, PC370, IMA and lactate after TBI modeling in rats. Such variation in the levels of oxidative stress biomarkers observed after TBI could be attributed to multidirectional differential mechanisms in their formation (lipid peroxidation for TBARS and oxidative protein modification for others) and discrepancies in the timing of biomarker post-injury assessment (immediate or delayed changes following TBI).

The administration of Eda reduced the oxidative stress biomarkers in serum, the levels of AOPP, PC370, PC430, and lactate were significantly decreased ($P < 0.05$) compared to the group of rats with TBI. Interestingly, protein carbonyl levels were even lower than in the group

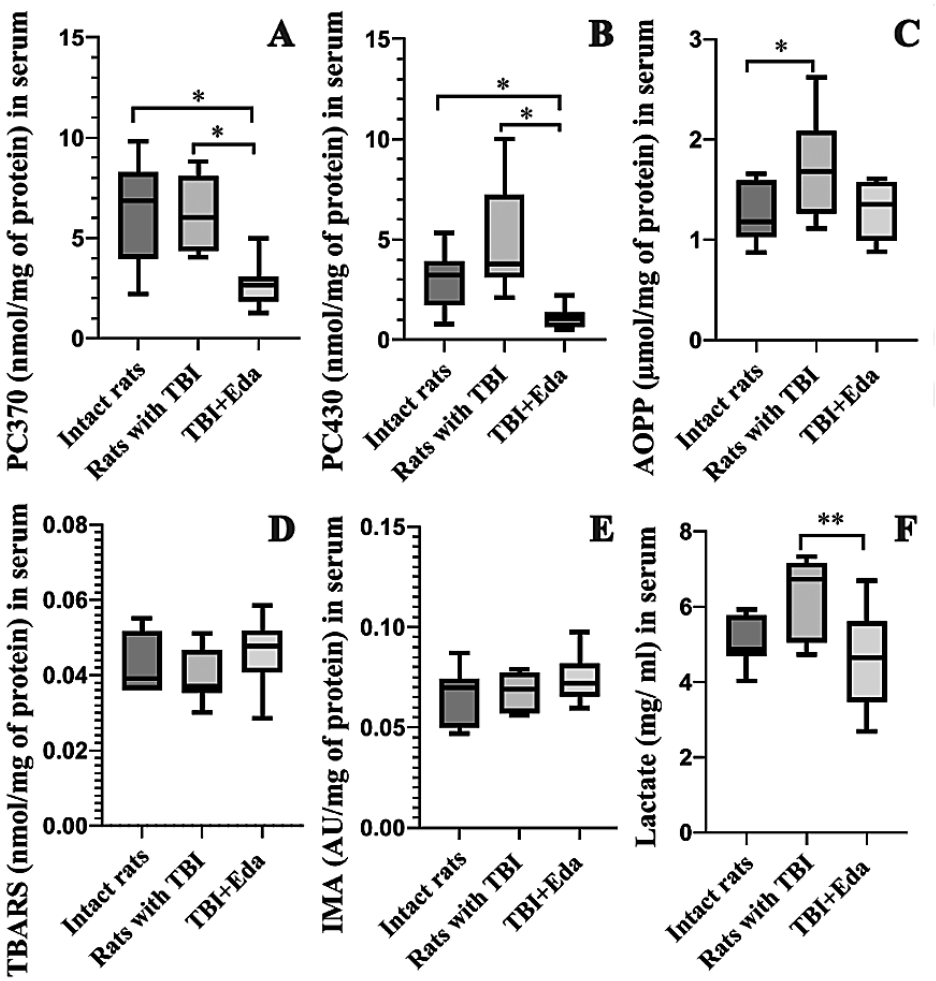

Figure 1. Oxidative stress biomarkers in serum
*Note. *P < 0.05, **P < 0.01.*

of intact animals. Based on these results, one can assume about launching drug-specific mechanisms for the rapid removal of oxidative products from the body after the use of Eda.

Moderate changes were observed in the S1 fraction of hippocampus and cerebral cortex, as shown in Figure 2 and Figure 3.

Despite the fact that TBARS levels had no significant changes after administration of edaravone, IMA level decreased significantly in the hippocampus. Interesting results were observed for lactate concentration, as it increased and was higher than in groups 1 and 2 in the hippocampus. Our data support the study by Thomas C. Glenn et al. [13] that the body increases lactate production to indirectly supply glucose to the injured brain. This work reported massive mobilization of body resources, mainly lactate, to support hepatic and renal gluconeogenesis. After isotopically labeled lactate was administered there were shown, that systemic lactate is directly consumed and used by the injured human brain and also that infusion of exogenous lactate can increase substrate supply to the brain while

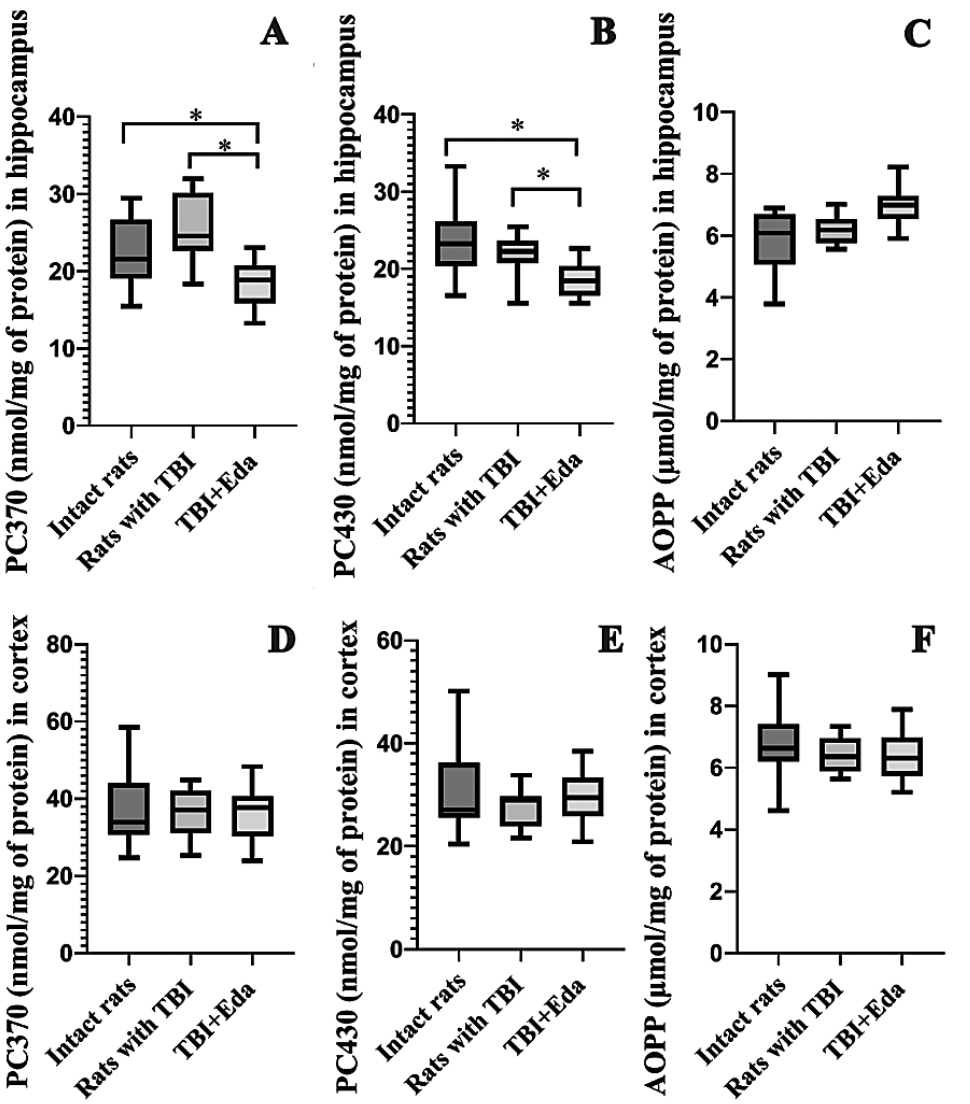

Figure 2. PC370, PC430 and AOPP in the fraction S1 of hippocampus and brain cortex
*Note. *P < 0.05.*

decreasing glycolysis, as occurs after TBI. We suggest that the use of a drug with antioxidant properties is a trigger for increased lactate production and helps to meet the nutrient needs of the injured brain.

Conclusion

According to the data obtained, the variously directed changes in biomarkers of carbonyl-oxidative stress in serum and brain indicate the complexity of oxidative damage caused by TBI at the systemic level. In our study, edaravone demonstrated effectiveness in reducing the levels of some specific oxidative stress biomarkers in rats with TBI. Specifically, it decreased the elevated PC370, PC430, AOPP and lactate in serum, indicating its ability to attenuate oxidative damage induced by TBI systemically. In addition to

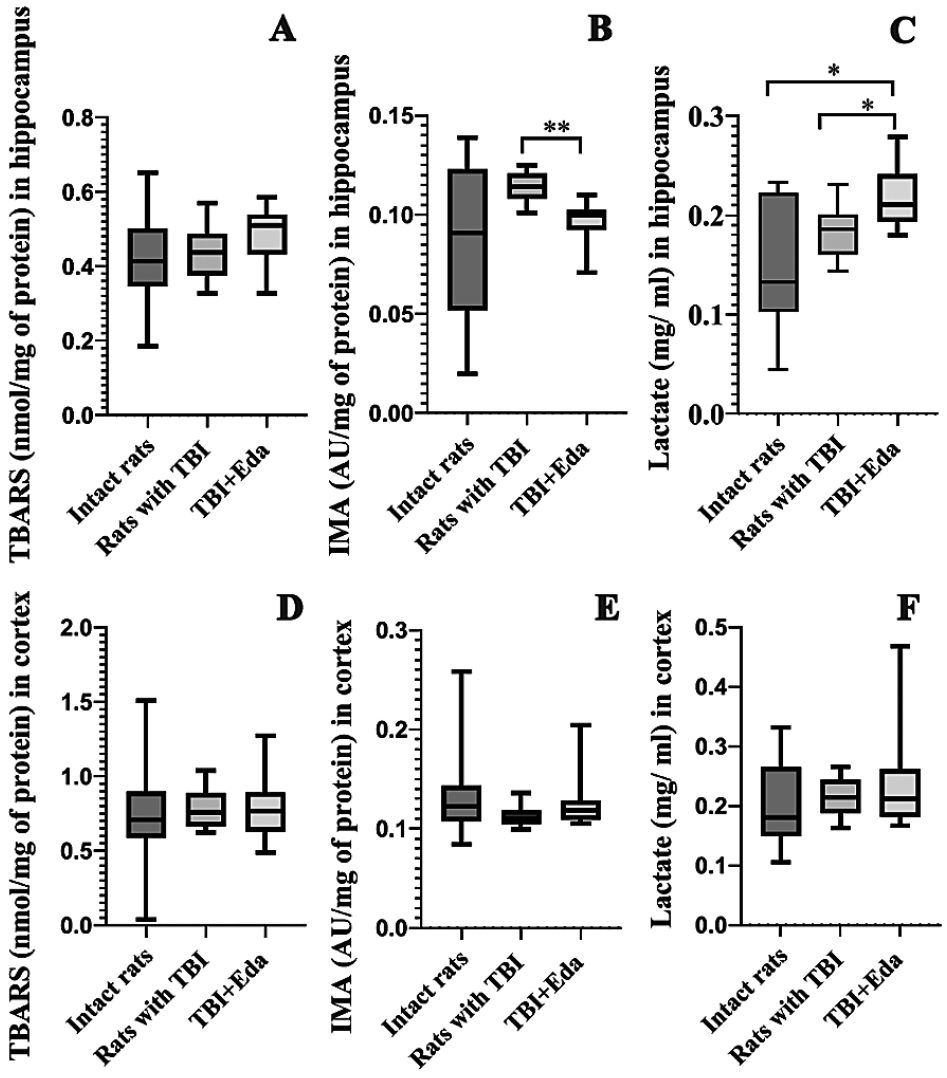

Figure 3. TBARS, IMA and lactate in the fraction S1 of hippocampus and brain cortex
*Note. *P < 0.05.*

systemic effects, edaravone had significant effects on protein carbonyls (PC370, PC430), IMA, and lactate in the hippocampus. These results provide valuable insight into the potential therapeutic effects of edaravone in TBI, but further studies are needed to elucidate the mechanisms of action of this drug. Long-term studies evaluating the functional outcomes, histopathologic changes and potential neuroprotective effects of edaravone treatment in TBI models are needed to confirm the therapeutic efficacy of this drug.

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It is known that traumatic brain injury (TBI) is one of the main causes of death and disability among people of all ages. Recently, the risk of TBI has increased dramatically in Ukraine, not only for the military, but also for civilians. After traumatic brain injury a cascade of multidirectional metabolic changes begins in the brain, including protein carbonylation, increased lipid peroxidation, free radicals formation, impaired neurotransmitters release and energy imbalance, which are associated with the development of various functional disorders. The search for effective drugs to eliminate or prevent these complications is very important. Nowadays, although edaravone has a limited range of indications (alleviation of neurological symptoms and functional disorders associated with acute ischemic stroke, as well as slowing the progression of functional disorders in amyotrophic lateral sclerosis), the known mechanisms of its pharmacological action may be useful in other conditions of the central nervous system, where oxidative stress plays a leading role in pathogenesis.

The aim of this study was to examine the effect of edaravone on biomarkers of carbonyl-oxidative stress in rats with TBI.

Modeling of TBI was carried out by applying a directed mechanical injury to the area of the scalped skull with a metal bar (450 g) falling from a height of 170 cm. Advanced oxidation protein products (AOPP), thiobarbituric acid reactive substances (TBARS), protein carbonyls (PC370, PC430), the content of lactate and ischemia modified albumin (IMA) in serum and S1 fractions of the cerebral cortex and hippocampus of experimental animals were studied using spectrophotometry.

It was established, that the modeling of TBI was associated with an elevation of PC430, AOPP and lactate in serum. Simultaneously, the levels of TBARS, IMA and lactate also were increased in the brain homogenates in addition to the above-mentioned changes. In our study edaravone demonstrated efficacy in reducing the levels of some specific biomarkers of oxidative stress. In particular, the administration of the drug was accompanied by a decrease in elevated contents of PC430, AOPP and lactate in serum, indicating its ability to systemically attenuate oxidative damage. It should be noted that a characteristic feature of the drug in case of TBI was its effect on the course of oxidative stress in the hippocampus. In addition, the ability of edaravone to a moderate increase in lactate production can be explained not by the phenomena of lactic acidosis, but by the activation of glia to meet the energy needs of the neurons in the injured brain.

The results obtained provide valuable insight into the potential therapeutic effects of edaravone in TBI, but require further studies to elucidate more detailed mechanisms of therapeutic action of this drug.

Key words: traumatic brain injury, biomarkers, oxidative stress, edaravone, brain, serum

В. А. Ткаченко, А. І. Шевцова, А. Е. Лєвих, Ю. В. Харченко, В. І. Жилюк

Дослідження впливу едаравону на біомаркери оксидативного стресу в сироватці крові та мозку щурів із черепно-мозковою травмою

Відомо, що черепно-мозкова травма (ЧМТ) є однією з основних причин смертності та інвалідності серед осіб різного віку. Останнім часом ризик отримати ЧМТ в Україні різко зріс не лише для військових, але й для цивільного населення. Після перенесеної ЧМТ у головному мозку починається каскад різноспрямованих метаболічних змін, що включають карбонілювання білків, посилення перекисного окиснення ліпідів, утворення вільних радикалів, порушення вивільнення нейромедіаторів, енергетичний дисбаланс, які пов'язані з розвитком різних функціональних розладів. Пошук ефективних препаратів для усунення чи профілактики цих ускладнень є дуже важливим. Сьогодні едаравон хоча й має обмежений спектр показів до застосування (полегшення неврологічних симптомів і функціональних розладів, пов'язаних з гострим ішемічним інсультом, а також сповільнення прогресування функціональних розладів при бічному аміотрофічному склерозі), проте відомі механізми його фармакологічної дії можуть бути корисними й у разі інших патологічних станів центральної нервової системи, де провідну роль відіграє окисний стрес.

Мета дослідження – вивчення впливу едаравону на біомаркери карбоніл-оксидативного стресу в щурів із ЧМТ.

Відтворення ЧМТ здійснювали шляхом нанесення спрямованого механічного удару на ділянку скальпованого черепа металевим бруском (450 г), що падав з висоти 170 см. Продукти окисної модифікації протеїнів (AOPP), реактивні речовини, що реагують з тіобарбітуровою кислотою (TBARS), карбоніли білків (PC370, PC430), уміст лактату й модифікованого ішемією альбуміну (IMA) в сироватці крові та фракціях S1 кори головного мозку та гіпокампу експериментальних тварин досліджували за допомогою спектрофотометрії.

Встановлено, що формування ЧМТ було асоційоване з підвищенням рівнів PC430, AOPP і лактату в сироватці крові. Водночас у гомогенатах головного мозку крім зазначених змін також зростали значення показників TBARS, IMA та лактату. У нашому дослідженні едаравон продемонстрував ефективність у вигляді зниження рівнів деяких специфічних біомаркерів оксидативного стресу. Зокрема, введення препарату супроводжувалося зниженням підвищених рівнів PC430, AOPP і лактату в сироватці крові, що вказує на його здатність системно послаблювати оксидативне пошкодження. Слід зазначити, що характерною особливістю препарату за ЧМТ був спрямований вплив на перебіг окисного стресу в гіпокампі. Окрім цього спроможність едаравону сприяти помірному збільшенню продукції лактату можна пояснити не явищами лактатацидозу, а активацією глії для задоволення потреб нейронів ушкодженого мозку в енергії.

Отримані результати дають цінне уявлення про потенційні терапевтичні ефекти едаравону при ЧМТ, проте вимагають подальших досліджень для з'ясування більш детальних механізмів терапевтичної дії цього препарату.

Ключові слова: черепно-мозкова травма, біомаркери, оксидативний стрес, едаравон, мозок, сироватка крові

ORCID ID співавторів:
Ткаченко В. А. (ORCID ID 0000-0003-2231-2913);
Шевцова А. І. (ORCID ID 0000-0003-0409-6030);
Лєвих А. Е. (ORCID ID 0000-0002-0615-5092);
Харченко Ю. В. (ORCID ID 0000-0002-4136-3495);
Жилюк В. І. (ORCID ID 0000-0003-2259-0622).

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Контактна особа: Шевцова А. І., Дніпровський національний університет імені Олеся Гончара, буд. 72, просп. Науки, м. Дніпро, 49045.Тел.: + 38 0 56 374 98 00.
Електронна пошта: paronic@ukr.net