UDC 616.831-005.1-002.1:616.379-008.64-092.9-07-085
https://doi.org/10.15407/biotech17.02.081

THE IMPACT OF EDARAVONE ON THE MARKERS OF CARBONYL-OXIDATIVE STRESS IN RATS WITH TRAUMATIC BRAIN INJURY

V.A. TKACHENKO[1], *A.I. SHEVTSOVA*[2], *A.E. LIEVYKH*[1], *Yu.V. KHARCHENKO*[1], *V.I. ZHYLIUK*[1]

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

E-mail: paronic@ukr.net

Received 2024/03/28
Revised 2024/04/16
Accepted 2024/04/30

Traumatic brain injury (TBI) is one of the leading causes of death and disability in people of all ages. The risk of receiving this kind of mechanical damage in wartime has greatly increased. After TBI, the brain develops a series of complex metabolic changes, including protein carbonylation, enhanced lipid peroxidation, impaired neurotransmitter release, imbalanced energy supply, that are associated with the development of different functional disorders [1]. Taking into account that there is still no adequate treatment to mitigate the devastating effects of carbonyl-oxidative stress (COS) after TBI on the metabolism in the central nervous system (CNS), the search for new drugs is still relevant.

Recently, Edaravone (Eda) has been widely used to treat cerebral infarction, based on its properties of highly effective elimination of free radicals, due to its high lipid solubility and permeability through the blood-brain barrier (BBB). Aside from its hydroxyl radical scavenging effect, Eda has been found to have beneficial effects on inflammation, nitric oxide production and apoptotic processes. Concordantly, Eda has demonstrated potent neuroprotective effects in a number of animal models of disease, including stroke, spinal cord injury, neurodegenerative diseases and brain tumors [2].

The *aim* of this comparative research was to study the effects edaravone on the markers of carbonyl-oxidative stress in rats with traumatic brain injury.

Methods. 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 head of a rat [3]. During TBI modeling, rats were under general anesthesia (zolazepam with tiletamine 20 mg/kg, intraperitoneally). Treatment with edaravone 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. 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). Advanced oxidation protein products (AOPP), thiobarbituric acid reactive substances (TBARS), protein carbonyls (PC370/PC430) were studied using spectrophotometry [4, 5] in serum and fraction S1 in brain cortex and hippocampus of experimental animals.

Results and Discussion. As shown in Table 1, the levels of PC430 and AOPP in the 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 not significant changes in the serum levels of TBARS and PC370 after TBI modeling in rats. Such variation in the levels of oxidative stress biomarkers observed after TBI could be attributed to 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 COS markers in the serum, the levels of AOPP, PC370 and PC430 were significantly

Table 1

Markers of COS in the serum

Markers of COS	Intact rats	Rats with TBI	TBI+Eda
TBARS (nmol/ml)	3.40±0.222	3.13±0.210	3.60±0,198
PC370 (nmol/mg of protein)	6.26±0.859	6.32±0.739	2.63±0.337*§
PC430 (nmol/mg of protein)	2.99±0.503	4.98±0.960	1.10±0.162*§
AOPP (μmol/mg of protein)	1.26±0.095	1.72±0.192§	1.28±0.088*

§ — $P < 0.05$ vs Intact rats; * — $P < 0.05$ vs Rats with TBI.

decreased ($P < 0.05$) compared to the group of rats with TBI. Interestingly, protein carbonyls levels were even lower than in the group of intact animals.

Moderate changes were observed in fractions S1 of the hippocampus and brain cortex, which are shown in Table 2. Minor effects were primarily observed in the fraction S1 of the hippocampus, where AOPP, TBARS, PC370 were increased in rats with TBI, but these changes were not significant. Brain tissues may activate neuroprotective mechanisms in response to TBI, including antioxidant enzyme induction, which could mitigate oxidative damage and maintain cellular homeostasis. These protective mechanisms counteract and attenuate oxidative stress-induced changes in biomarker levels. It should be noted that in the group TBI+Eda, there was a significant decrease in the values of PC370 by 28% ($P < 0.05$) and PC430 by 14% ($P < 0.05$) in the hippocampus S1 fraction compared to the group of rats with TBI. These biomarkers were even lower than in the group of intact animals. Simultaneously, there were no significant changes of COS markers in the brain cortex of rats in all experimental groups.

Table 2

Markers of COS in fractions S1 of the hippocampus and brain cortex

Markers of COS	Intact rats	Rats with TBI	TBI+Eda
Fraction S1 (hippocampus)			
TBARS (nmol/ml)	2.16±0.206	2.720±0.176	3.365±0.141
PC370 (nmol/mg of protein)	22.49±1.452	25.33±1.608	18.39±0.937*§
PC430 (nmol/mg of protein)	23.67±1.628	21.74±1.030	18.72±0.744*§
AOPP (μmol/mg of protein)	5.83±0.357	6.19±0.170	6.96±0.195
Fraction S1 (brain cortex)			
TBARS (nmol/ml)	4.313±0.481	4.979±0.262	6.661±0.220
PC370 (nmol/mg of protein)	37.55±3.615	36.30±2.364	36.52±5.028
PC430 (nmol/mg of protein)	30.81±2.071	27.55±1.415	29.42±0.428
AOPP (μmol/mg of protein)	6.53±0.301	6.40±0.207	6.36±0.260

§ — $P < 0.05$ *vs* Intact rats; * — $P < 0.05$ *vs* Rats with TBI.

Conclusions. Overall, the discrepancy in oxidative stress biomarker responses between serum and brain tissue homogenates highlights the complexity of oxidative stress dynamics in the context of TBI and underscores the importance of considering multiple factors when interpreting experimental findings. 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 levels of PC430 and AOPP in the serum, indicating its ability to attenuate oxidative damage induced by TBI systemically. In addition to its systemic effects, Edaravone exerted targeted effects on COS-modified proteins within the hippocampus, even though these biomarkers were not significantly altered by TBI alone. While these findings provide valuable insights into the potential therapeutic effects of Edaravone in TBI, further research is needed to elucidate the underlying mechanisms of this drug. Long-term studies assessing functional outcomes, histopathological changes, and potential neuroprotective effects of Edaravone treatment in TBI models are warranted to validate its therapeutic efficacy.

Key words: traumatic brain injury, oxidative modification of proteins, carbonyl-oxidative stress, edaravone.

Authors' contribution. AEL, VIZ, and AIS conceived and designed this research, organized, performed, and carried out the experiment; VAT, YVK conducted the experiments and the statistical processing of results.

Funding source. The work was carried out within the framework of the State budget theme of the Department of Pharmacology of Dnipro State Medical University (2023-2026, State registration No. 0123U100028).

REFERENCES

1. *Lai J., Shi Y., Lin S., Chen X.* Metabolic disorders on cognitive dysfunction after traumatic brain injury. *Trends Endocrinol. Metab.* 2022, 33 (7):451-462. https://doi.org/10.1016/j.tem.2022.04.003
2. *Shakkour Z., Issa H., Ismail H., et al.* Drug repurposing: promis-es of edaravone target drug in traumatic brain injury. *Curr Med Chem* 2021;28:2369-91. https://doi.org/10.2174/0929867327666200812221022
3. *Marmarou C.R., Prieto R., Taya K., Young H.F., Marmarou Y., Marmarou A.* Weight Drop Injury Model. In: Chen J., Xu Z.C., Xu XM., Zhang J.H. (eds) *Animal Models of Acute Neurological Injuries. Springer Protocols Handbooks. Humana Press.* 2009;34:393–407. https://doi.org/10.1007/978-1-60327-185-1_34
4. *Lievykh A., Zhyliuk V., Tkachenko V., Kharchenko Yu., Kovalchuk Yu., O. Dovban, Ushakova G., Shevtsova A.* Impact of Vitamin D3 on Carbonyl-Oxidative Stress and Matrix Metalloproteinases after Acute Intracerebral Hemorrhage in Rats with Type 2 Diabetes Mellitus. Endocr Metab Immune Disord Drug Targets. 2023; 23(10):1326-1339. https://doi.org/10.2174/1871530323666230321100534
5. *Diaz De Leon J.A., Borges C.R.* Evaluation of Oxidative Stress in Biological Samples Using the Thiobarbituric Acid Reactive Substances Assay. *J Vis Exp.* 2020; 12:(159):10.3791/61122. https://doi.org/10.3791/61122