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EDARAVONE REDUCES THE MARKERS OF OXIDATIVE STRESS AND NEUROINFLAMMATION IN NEOCORTEX OF RATS WITH ACUTE INTRACEREBRAL HEMORRHAGE AND TYPE 2 DIABETES MELLITUS

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Type 2 diabetes mellitus (T2DM) is associated with a higher incidence of hemorrhagic stroke in a severe form. The aim of this study was to estimate the markers of oxidative stress and neuroinflammation in the brain of rats with acute intracerebral hemorrhage (ICH) and T2DM after treatment with edaravone. T2DM was induced by a single intraperitoneal injection of nicotinamide/streptozotocin, ICH – by stereotactic microinjection of bacterial collagenase. Rats were randomized into four groups: 1 – intact control; 2 – T2DM; 3 – T2DM+ICH; 4 – T2DM+ICH+edaravone 6 mg/kg/day. Edaravone (a drug to treat neural injury after acute cerebral ischemic stroke) was administered intraperitoneally for 10 days starting from the 60th day after diabetes mellitus induction and 30 min after ICH induction. Brain homogenates were assessed for the content of advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs). The levels of TNF-α and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured with ELISA. The increased content of 8-OHdG and TNF-α in brain homogenates of animals of T2DM group compared to the control was shown. It was revealed that in brain homogenates of animals of T2DM+ICH group the content of these markers significantly exceeds that for T2DM group, and in addition, an elevated AOPPs level was observed. Our results demonstrated that edaravone prevented the elevation of TNF-α level, reduced oxidative DNA damage by decreasing 8-OHdG content, and attenuated the formation of AGEs and AOPPs in the brains of experimental animals. These findings suggest that edaravone may have therapeutic potential in diabetic patients with acute ICH.

K e y w o r d s: edaravone, intracerebral hemorrhage, type 2 diabetes mellitus, TNF-α, neuroinflammation, oxidative stress.

Spontaneous intracerebral hemorrhage (ICH) is a serious cerebrovascular condition leading to high mortality and morbidity in adults that accounts for 10-30% of all strokes and affects more than one million people worldwide every year. Compared to ischemic stroke, hemorrhagic stroke leads to a higher mortality rate, increased need for palliative care, and higher hospitalization costs. The extravasated blood accumulates and compresses the surrounding brain tissues forming a hematoma, the components (especially the blood-derived leukocytes) of which infiltrate the brain parenchyma and disrupt the blood-brain barrier (BBB) leading to inflammatory responses and cerebral edema as well as neuronal damage [1-3]. The 5-year mortality rate remains very high (52% for males, 56% for females) in ICH patients older than 45 years. Even after surgical treatment, 20% of these patients experience varying degrees of neurological dysfunction, requiring long-term hospitalization and rehabilitation [4].

Diabetes mellitus is a major public health concern and a continuously growing chronic disease worldwide. Diabetes mellitus is one of the modifiable, independent, and major risk factors of ischemic stroke. Moreover, type 2 diabetes mellitus (T2DM) accounts for almost 90% of the approximately 537 million recorded cases of diabetes worldwide. The number of diabetic patients is constantly increasing, and the trend among children and young people under the age of 40 is particularly alarming

[5]. The global prevalence of prediabetes and diabetes has led to a corresponding epidemic of diabetic complications. Traditional studies on the pathogenesis of these complications have largely focused on risk factors, such as hyperglycemia, oxidative stress, inflammation, advanced glycation end products (AGEs), activation of stress kinases, protein kinase C (PKC), and others. Currently, it is recommended that macro/micro-vascular complications of diabetes be distinguished from parenchymal ones. [6].

Diabetes mellitus is a significant risk factor for brain injury. Brain injury is associated with acute and chronic hyperglycaemia, insulin resistance, hyperinsulinemia, diabetic ketoacidosis (DKA) and hypoglycaemic events in diabetic patients. The pathogenesis of hyperglycemia-induced brain injury is complex. It includes a combination of vascular disease, oxidative stress, neuroinflammation, neurodegeneration, mitochondrial dysfunction, apoptosis, reduction of neurotrophic factors, acetylcholinesterase (AChE) activation, neurotransmitter changes, deficiency of brain repair processes, impairment of brain lymphatic system, accumulation of amyloid β and tau phosphorylation [7]. Type 2 diabetes mellitus is associated with a higher incidence of hemorrhagic stroke, and patients with T2DM have more severe hemorrhagic strokes (higher NIHSS) at admission and discharge, which contributes to a larger percentage with unfavorable discharge outcomes [3, 8].

Constituting less than 2% of the body, the brain uses over 20% of the oxygen delivered to the organism. Phospholipids of cerebral cell membranes are also enriched in polyunsaturated fatty acids which, together with the low activity of brain antioxidant enzymes and the high content of prooxidant metal ions (e.g., Fe^{2+}, Cu^{2+}, Co^{2+}, and Cr^{2+}), make the brain very vulnerable to oxidative stress [9]. Substantial evidence generated from the experimental models of diabetes indicates that hyperglycemia and its metabolic derangements result in oxidative stress, abnormal lipid peroxidation, impaired antioxidant defenses, and subsequent necrotic and apoptotic neuronal cell death [7].

Oxidative stress (OS) also plays an important role in brain injury after ICH. Upon bleeding into the parenchyma, elevated glutamate, infiltrating inflammatory cells, and the metabolic products of erythrocyte lysis are the sources of active free radical generation. Free radical overproduction is accompanied by prooxidase activation and antioxidase inhibition, causing OS in ICH. The direct biomolecule oxygenation and indirect cell death signaling pathway activation by reactive oxygen species (ROS)/reactive nitrogen species (RNS) are responsible for OS-induced brain damage after ICH [10]. The mechanisms involved in these prooxidant effects include endoplasmic reticulum (ER) stress, neuronal apoptosis and necrosis, inflammation, and autophagy [4]. The use of antioxidant compounds can likely block the sources of oxidative stress in ICH and diabetes mellitus and neutralize the existing excessive production of free radicals. This may reduce mortality and improve recovery in patients with ICH, especially with such comorbid pathology as diabetes mellitus.

Edaravone is an exogenous scavenger of hydroxyl radicals (˙OH) that inhibit both ·OH-dependent and ˙OH-independent lipid peroxidation [11]. A lot of experimental studies suggest that edaravone exerts its neuroprotective effects by increasing mature brain-derived neurotrophic factor (mBDNF) and Bcl-2 expression, decreasing caspase-3 activity, and promoting extracellular signal-regulated kinases 1/2 (ERK1/2) activation in cultured neurons [12]. Edaravone has also been shown to reduce propofol neurotoxicity in the hippocampus by reducing propofol-induced inhibition of proliferation, neuroapoptosis, and neuroinflammation by activating the mBDNF/TrkB/PI3K pathway [13].

The drug was first used clinically in 2001 to treat neural injury after acute cerebral ischemic stroke [13, 14]. Currently, the indications for its use are acute ischemic stroke and amyotrophic lateral sclerosis. Although official indications are limited to the conditions mentioned above, edaravone has recently attracted the attention of researchers due to its effectiveness in other diseases, such as multiple sclerosis, oncological pathology, certain viral infections, and Alzheimer's disease [15-18]. To date, there is limited data on the efficacy of edaravone in the conditions of hemorrhagic brain injury.

According to a recent meta-analysis based on 38 randomized controlled trials, the use of edaravone within 7 days of the onset of the disease improved neurological deficits and daily activities of the patients. Also, it was associated with a reduction in hematoma volume but did not reduce patient mortality. Nevertheless, the results of this meta-analysis are insufficient to support the use of edaravone for the routine treatment of acute ICH [19]. Therefore, further studies of edaravone as a neuroprotective therapy for ICH are relevant. Its unique mechanism of action towards oxidative stress may be useful not only in

acute or chronic diseases of the nervous system but also in chronic comorbidities, in particular, diabetes mellitus. The latter is accompanied by excessive production of free radicals and the development of oxidative stress, which is the main pathogenetic link in the development of complications in this pathology. Diabetes mellitus has a synergistic negative effect on the state of neurons and glial cells and, thus, may initiate the onset or intensify the complications of ICH.

The aim of this study was to compare the effects of edaravone on the markers of oxidative stress and neuroinflammation in the brain of rats with acute intracerebral hemorrhage and type 2 diabetes mellitus.

Materials and Methods

Studied drugs. Edaravone (Xavron®, Yuria-Pharm, Ukraine; Eda, 6 mg/kg/day) was used in the study.

Experimental animals and groups. 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 the Dnipro State Medical University (protocol N8 dated 17.12.2019). 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. According to the result of the oral glucose tolerance test, all rats with a similar degree of glycemia were randomly divided into four groups: group 1 – intact control/naive (saline, 5 ml/kg/day, n = 8); group 2 – pathology control A (T2DM+saline, n = 9); group 3 – pathology control B (T2DM+ICH+saline, n = 7); group 4 – animals that received edaravone 6 mg/kg/day (T2DM+ICH+Eda, n = 6).

The first administration of edaravone was carried out 30 min after the induction of ICH by intracerebral microinjection of collagenase. The studied drug was administered into the peritoneal cavity once daily for 10 days, starting from the 60th day after the induction of diabetes mellitus. Rats of the intact control and pathology control groups A and B received saline 5 ml/kg/day by intraperitoneal injection.

Type 2 diabetes mellitus (T2DM) was induced by a single intraperitoneal injection of nicotinamide 230 mg/kg (NA, Sigma-Aldrich, USA) and streptozotocin 65 mg/kg (STZ, AdooqBioscience, USA) in citrate buffer (pH = 4.5, 0.1 M) to overnight fasted rats [20]. Blood glucose level was measured 72 h after NA/STZ injection. Animals with values less than 8.3 mmol/l were excluded from the study.

Glucose level was measured using the blood glucose meter Bionime Rightest GM300 (Bionime Corporation, Switzerland) in blood samples obtained from the tail vein. The oral glucose tolerance test (OGTT) was performed on the 69th day of the study. Overnight fasted animals were given glucose 2 g/kg (20% solution) by intragastric gavage 2 h after drug administration. The area under the glycemic curve (AUC) was calculated using GraphPad Prism 8.0 software and expressed as min×mmol/l.

ICH in rats was induced by microinjection of 1 µl of bacterial collagenase 0.2 IU/µl (Type IV-S, Sigma-Aldrich, USA) [21]. On the 60th day after NA/STZ injection, all rats were anesthetized with the intramuscular injection of tiletamine (15 mg/kg, Zoetis, Spain), zolazepam (15 mg/kg, Zoetis, Spain), xylazine (5 mg/kg, Interchemie, Holland) and then placed in a stereotaxic frame. A Hamilton microsyringe was inserted into the striatum of rats by the following stereotactic coordinates: 0.2 mm anterior, 2.8-3.0 mm lateral, and 5.5 mm ventral to the bregma.

Preparation of brain. On the 70th day of the study, all rats were anesthetized and sacrificed. Their brains were quickly removed, perfused with ice-cold phosphate-buffered saline (PBS, Sigma-Aldrich, USA), washed, dried, and dissected to separate the cortex. Specimens from every brain were separated, weighed, and stored at −45°C for further analyses.

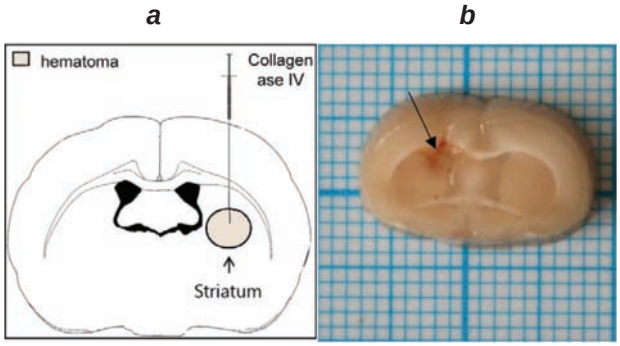

*Fig. 1. Schematic representation of the method for ICH induction (**a**). Representative image of brain coronal section (straight-cut at the injection site) in group 3 (T2DM+ICH+saline) (**b**)*

In addition, all brain samples from the ICH-affected hemisphere were collected and kept in 10% neutral buffered formalin for histopathological examination.

The first half of cortical tissues was homogenized (5% w/v) in 20 mM phosphate buffer (pH 7.4) and centrifuged at 10 000 g for 10 min at 4°C. Obtained supernatants were used to measure the levels of advanced glycated end products (AGEs), advanced oxidation protein products (AOPPs) and lactate. Another half of cortical tissues were homogenized (10% w/v) in a buffer containing 25 mM Tris-HCl (Sigma-Aldrich, USA) pH 7.4, 1 mM EDTA (Sigma-Aldrich, USA), 2 mM β-mercaptoethanol (Sigma-Aldrich, USA), 0.2 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich, USA) and 0.01% merthiolate (Sigma-Aldrich, USA). The homogenates were centrifuged at 10 000 g for 10 min (4°C), and the supernatants were used for enzyme-linked immunosorbent assay (ELISA).

Biochemical analysis. Glycated hemoglobin (HbA1c) was measured spectrophotometrically in whole blood samples using a standard HbA1c kit (Reagent, Ukraine). The principle of the method is based on the presence of 1-deoxy-1(N-valyl) fructose in a stable form of HbA1c. Further, it is dehydrated by phosphoric acid to 5-hydroxymethyl-2-furaldehyde, which forms a color complex with 2-thiobarbituric acid with maximum absorption at 443 nm [22]. The content of HbA1c was expressed as μmol fructose/g Hb.

The level of advanced glycated end products (AGEs) was measured by fluorescence method [23], using a Hoefer DQ 2000 Fluorometer (USA) with fixed wavelengths (excitation/emission = 365/460 nm). The results were expressed as arbitrary units (AU) per mg protein.

The level of advanced oxidation protein products (AOPPs) was measured using spectrophotometry on a microplate reader, according to the modified method described by Witko-Sarsat [24, 25]. The AOPPs were calibrated with chloramine-T solutions that absorb at 340 nm in the presence of potassium iodide. Briefly, the samples were distributed on a standard 96-well microtiter plate, and 30 μl of 50% acetic acid (Sigma-Aldrich, USA) was added. In standard wells, 15 μl of 1.16 M potassium iodide (Sigma-Aldrich, USA) was added to 200 μl of chloramine-T solution 0-100 μmol/l (Sigma-Aldrich, USA), followed by 30 μl of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm on the microplate reader against a blank well containing 200 μl of PBS, 15 μl of potassium iodide, and 30 μl of acetic acid. Because the absorbance of chloramine-T at 340 nm is linear up to 100 μmol/l, AOPPs levels were expressed as μmol chloramine-T equivalents per mg of protein (μmol/mg protein).

Enzyme-linked immunosorbent assay (ELISA). The assays of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and tumor necrosis factor-alpha (TNF-α) in brain homogenates were performed according to the manufacturer protocols (ELISA kits E-EL-0028 and E-EL-R2856, Elabscience, USA).

Statistical analysis. GraphPad Prism software (version 8, San Diego, CA, USA) was used for statistical analysis of the results. Before applying the statistical criteria, the hypothesis of the normal distribution of random variables was tested (according to the Shapiro-Wilk test). All results are expressed as mean ± SD. Statistical significance ($P < 0.05$) was determined by a two-tailed Student's *t*-test or one-way ANOVA for normally distributed variables, and Mann-Whitney U-test or Kruskal-Wallis H-test for non-normally distributed variables.

Results

The study of blood glucose levels showed that the development of experimental T2DM in rats led to a significant increase in basal glycemia, but comorbid ICH did not affect its severity (Table 1). In addition, the results of OGTT showed significant changes in blood glucose AUC in all experimental groups versus intact animals. According to the data obtained, the course of T2DM (group 2) led to the development of glucose tolerance, as evidenced by an increase in glycemic AUC by 58.1% ($P < 0.01$). Simultaneously, ICH modeling (group 3) did not increase the level of glucose tolerance in this test, and AUC was higher by 75.3% ($P < 0.01$) as compared to group 1, but there were no significant changes between groups 2 and 3. It must be noted that edaravone did not have any considerable hypoglycemic effect.

The rats in group 2 had increased HbA1c levels by 37.4% ($P < 0.05$) as compared to the animals in group 1 on the 69th day of the experiment. Moreover, this marker was higher by 54.2% ($P < 0.01$) in rats with T2DM and ICH (group 3) as compared to group 1. Edaravone slightly reduced HbA1c levels by 18.3% ($P < 0.05$) in comparison with group 3 (Table 1).

It was found that acute ICH in rats with T2DM (group 3) significantly did not affect the levels of

Table 1. Effects of edaravone on glucose homeostasis in rats with T2DM and ICH (mean ± SD)

Experimental groups	Glucose, mmol/l	HbA1c, μmol fructose/g Hb	AUC, min×mmol/l
Group 1 – Intact control (saline, 5 ml/kg/day, *n* = 8)	5.25 ± 1.28	1.31 ± 0.32	754.70 ± 212.21
Group 2 – Pathology control A (T2DM+saline, *n* = 9)	6.92 ± 1.76*	1.80 ± 0.32*	1193.20 ± 304.75**
Group 3 – Pathology control B (T2DM+ICH+saline, *n* = 7)	6.59 ± 0.35*	2.02 ± 0.36**	1323.00 ± 206.91**
Group 4 – Edaravone 6 mg/kg/day, (T2DM+ICH+Eda, *n* = 6)	6.90 ± 1.40*	1.65 ± 0.23*#	1124.80 ± 222.57**

Note. *$P < 0.05$, **$P < 0.01$ (vs. intact control); #$P < 0.05$ (vs. pathology control B)

AGEs in homogenates of the cerebral cortex as compared to group 2, even though this marker was slightly higher by 18.5% ($P < 0.05$) than in the intact control group (Fig. 2, *a*). In addition, there was a considerable increase in the AOPPs level by 53.1% ($P < 0.001$) in rats with comorbid pathology vs. group 1 and by 27.2% ($P < 0.01$) vs. group 2, which indicates activation of oxidative stress due to hemorrhagic lesions of the brain (Fig. 2, *b*). Edaravone significantly reduced both AGEs and AOPPs levels in the brain homogenates by 20.7% ($P < 0.01$) and 28.3% ($P < 0.01$) respectively.

A dynamic increase in the 8-OHdG level by 48,6% ($P < 0.05$) was also found in group 2, and it was further elevated in group 3 by 90.9% ($P < 0.001$) vs. intact rats (Fig. 2, *c*). Administration of edaravone provided a significant reduction of this marker by 23.3% ($P < 0.05$) vs. group 3 (Fig. 2, *c*).

Similar changes were noted for TNF-α levels (Fig. 2, *d*). It was found that the course of T2DM, both isolated and complicated by ICH, led to an increase of TNF-α in brain homogenates by 38.6% ($P < 0.05$) and 121.3% ($P < 0.01$) vs. intact rats respectively. At the same time, the administration of edaravone contributed to a significant reduction of TNF-α level by 46.3% ($P < 0.05$) as compared to group 3 (Fig. 3, *d*).

Discussion

It is known that elevated blood glucose levels worsen the breakdown of the BBB, increase brain water content, and lead to cellular apoptosis after ICH [26-28]. Diabetes mellitus further complicates the outlook for ICH by disrupting various mechanistic pathways involved in secondary brain injury, including neuroinflammatory processes, oxidative stress, vascular dysfunction, and systemic inflammation [29-31].

According to the data obtained, high levels of AOPPs, AGEs, 8-OHdG, and TNF-α indicate oxidative stress in the brain tissue in T2DM, which may play an important role in nerve tissue damage, cognitive dysfunction, and neurological deficits in this pathology [32]. Furthermore, we found that ICH significantly increases the severity of oxidative stress in the brain tissue of diabetic rats, which can have a negative effect on both neurons and endothelial cells of the brain microvasculature.

In our study, edaravone had a significant effect on the markers of both oxidative stress (AGEs, AOPPs and 8-OHdG) and neuroinflammation (TNF-α) in the brain. Such dual action indicates the positive effect of the drug on the course of free radical oxidation reactions intensified by both diabetes and ICH. The antioxidant properties of edaravone, as an exogenous free radical scavenger, have been known for a long time, and the current experimental study confirms its potential in the combined pathology T2DM+ICH. Hata et al. demonstrated role of edaravone in reducing hydroxyl radical-induced DNA damage, especially in repairing base lesions and abasic sites [33]. Also, a protective role of edaravone against radiation-induced oxidative damage was clearly established. This aligns with our findings that edaravone reduces oxidative stress markers 8-OHdG and AGEs, suggesting its broad capacity to counteract oxidative damage in various pathological conditions.

Simultaneously, a significant decrease in the levels of TNF-α in the brain tissue is another possible mechanism of the drug to suppress the manifestations of neuroinflammation in the combined

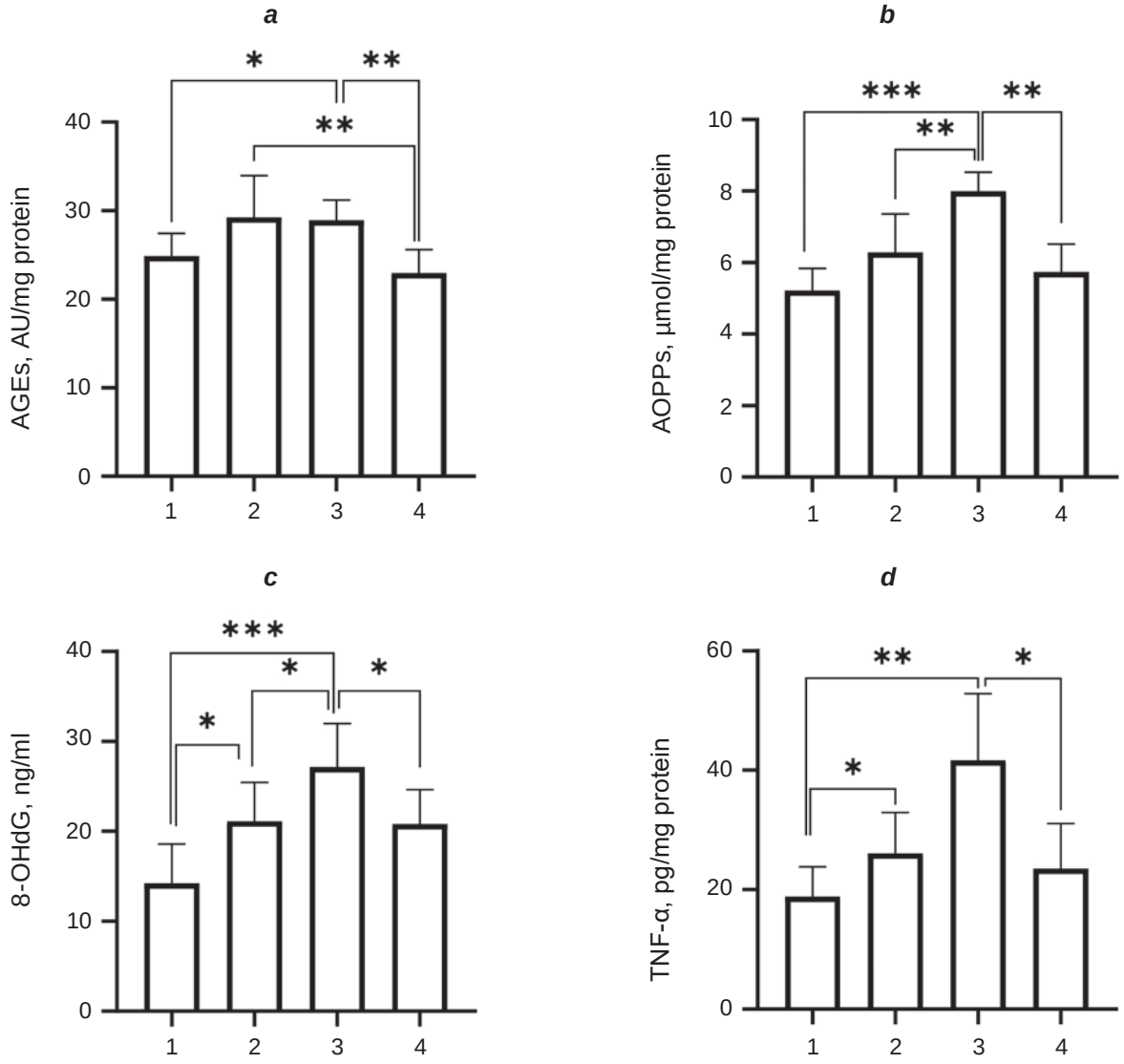

*Fig. 2. Levels of AGEs (**a**), AOPPs (**b**), 8-OHdG (**c**) and TNF-α (**d**) in the brain homogenates. Data are presented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001*

pathology T2DM+ICH. Tumor necrosis factor-alpha (TNF-α) is a master regulator of systemic and neuronal inflammatory processes. It is involved in the pathogenesis of numerous central nervous system disorders, including cerebral ischemia, Parkinson's disease, and traumatic brain injury (TBI). TNF-α levels are reported to become rapidly elevated post-TBI and, potentially, can lead to secondary neuronal damage. It is known that TNF-α is involved in the delayed neuronal cell death and gliosis that occurs following brain injury, which leads to cognitive deficits. At the same time, the pharmacological limiting of the elevation of TNF-α within 12 h after the brain injury event markedly reduces such secondary damage and leads to improved cognitive outcomes [34, 35]. An increase in perihematomal TNF-α levels contributes to brain edema formation after ICH [36]. Raised plasma concentration of tumor necrosis factor receptors (TNFR) has been linked with ICH incidents, most clearly with ICH of nonlobar location. The results suggest that TNF-α-mediated inflammation could be associated with vascular changes preceding ICH [37].

Stimulation of the production and activity of various inflammatory factors can likely be initiated not only by oxidative stress but also by TNF-α. The regulation of matrix metalloproteinases (MMPs), especially MMP-9, has a critical role in pathological

events in the central nervous system (CNS). MMP-9 is a marker of inflammation that triggers several CNS disorders, including neurodegeneration. Both experimental and clinical studies show that MMPs are upregulated after acute ICH. They are released into the area of brain injury by a variety of cells, including activated microglia, leukocytes, astrocytes, neurons, and endothelial cells. The expression of MMPs increases early after ICH: the brain and serum levels of MMPs peak within the first days and remain high across the first week after hematoma development. Overexpression of specific isoforms of MMPs plays a role in the development of secondary brain damage. By promoting the loss of vascular integrity, increasing the permeability of vascular walls, and activating inflammatory response and cellular death, MMPs contribute to the disruption of BBB, edema propagation, hematoma growth, and neuronal loss. Overall, there is substantially converging evidence from experimental studies to suggest that early and short-term inhibition of MMPs after ICH can be an effective strategy to reduce cerebral damage and improve the outcome, whereas long-term treatment may be associated with more harm than benefit [1, 38]. In our previous study, edaravone reduced oxidative modification of proteins and modulated the activity of MMP2 and MMP9 in the serum of rats with experimental T2DM complicated by collagenase-induced ICH [39]. This relationship can be explained by the fact that TNF-α, a key inflammatory cytokine, and oxidative stress are known to induce MMP transcription and activation [1]. TNF-α, alongside other pro-inflammatory cytokines such as IL-6, IL-1β, and IL-18, is involved in upregulating MMP expression [40]. Ability edaravone to reduce oxidative stress markers and inflammatory cytokines, shown in our current and previous studies, suggests that it may indirectly modulate MMPs through these pathways. The reduction in MMP activity could lead to decreased matrix degradation, highlighting the neuroprotective effect of edaravone in the combined pathology of T2DM and ICH. This complex interplay between oxidative stress, inflammation, and MMPs could explain how edaravone mitigates tissue damage and inflammatory responses.

In view of the growing interest in the role of TNF-α in initiating and modulating neuroinflammation, which may contribute to the progression of acute and chronic neurological disorders, our study highlights the value of targeting TNF-α as a treatment strategy for brain injury, particularly for neuroprotection after ICH.

Conclusions. Edaravone is highly effective in the reduction of oxidative stress and neuroinflammation in diabetic rats with intracerebral hemorrhage, which further emphasizes its neuroprotective properties and the possibility of using the drug in diabetic patients with hemorrhagic stroke. Moreover, edaravone provides neuroprotection not only due to antioxidant properties but also by inhibiting the synthesis of TNF-α.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ЕДАРАВОН ЗМЕНШУЄ МАРКЕРИ ОКСИДАТИВНОГО СТРЕСУ ТА НЕЙРОЗАПАЛЕННЯ В НЕОКОРТЕКСІ ЩУРІВ ІЗ ГОСТРИМ ВНУТРІШНЬОМОЗКОВИМ КРОВОВИЛИВОМ ТА ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ

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Цукровий діабет 2 типу (ЦД2) асоціюється з вищою частотою геморагічного інсульту у важкій формі. Метою цього дослідження було оцінити маркери оксидативного стресу та нейрозапалення в головному мозку щурів із гострим внутрішньомозковим крововиливом (ВМК) та ЦД2 після лікування едаравоном. ЦД2 спричинювали одноразовим внутрішньоочеревинним введенням нікотинаміду/стрептозоцину, ВМК – стереотаксичною мікроін’єкцією бактеріальної колагенази. Щурів рандомізували на чотири групи: 1 – інтактний контроль; 2 – ЦД2; 3 – ЦД2+ВМК; 4 – ЦД2+ВМК+едаравон 6 мг/кг/доба. Едаравон (препарат для лікування церебрального ішемічного інсульту) вводили

внутрішньоочеревинно впродовж 10 днів, починаючи з 60-го дня після індукції цукрового діабету та через 30 хв після індукції ВМК. Гомогенати головного мозку досліджували на вміст кінцевих продуктів глікації (КПГ) та кінцевих продуктів окислення протеїнів (КПОП). Рівні фактора некрозу пухлин-альфа (ФНП-α) та 8-гідрокси-2′-дезоксигуанозину (8-ОНдГ) вимірювали методом ІФА. Показано підвищення вмісту 8-ОНдГ та ФНП-α в гомогенатах головного мозку у тварин групи ЦД2 порівняно з контролем. Виявлено, що в гомогенатах головного мозку тварин групи ЦД2+ВМК вміст цих маркерів достовірно перевищив показники групи ЦД2, крім того, спостерігався підвищений рівень КПОП. Наші результати продемонстрували, що едаравон запобігав підвищенню рівня ФНП-α, зменшував окислювальне пошкодження ДНК шляхом зниження вмісту 8-ОНдГ і послаблював утворення КПГ і КПОП у головному мозку щурів. Зроблено висновок, що едаравон може мати терапевтичний потенціал у хворих на цукровий діабет із гострим ВМК.

Ключові слова: едаравон, внутрішньомозковий крововилив, цукровий діабет 2 типу, ФНП-α, нейрозапалення, оксидативний стрес.

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