Effects of edaravone on oxidative protein modification and activity of gelatinases after intracerebral hemorrhage in rats with nicotinamide-streptozotocin induced diabetes

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Abstract

Stroke, especially hemorrhagic form, is one of the most serious comorbidity disease of Diabetes Mellitus (DM), often associated with high mortality, particularly in Type 2 DM (T2DM). Therefore, it is relevant the search for drugs with a metabolically justified protective effect. Edaravone (Eda) is widely used for treating ischemic stroke, but its biochemical effects in Intracerebral Hemorrhage (ICH) associated with T2DM are still not confirmed. The aim of the study was to assess the impact of Eda on the markers of Oxidative Modification of Proteins (OMP), such as Advanced Oxidation Protein Products (AOPP), neutral and basic carbonyls (PC370 and PC430), Advanced Glycation End products (AGEs) and Ischemia Modified Albumin (IMA) as well as on the activity of matrix metalloproteinases MMP2/MMP9 (gelatinases) in rats with experimental T2DM after collagenase-induced ICH. Metformin was used as a comparative drug. The data obtained indicate that ICH in diabetic rats is accompanied by an increase in AOPP, PC370, AGEs, and mature forms of both gelatinases. On the contrary, IMA and proMMP9 were below normal level after ICH. Both studied drugs decreased the OMP markers to the levels of intact rats or lower, and Eda demonstrated a more potent effect. Besides, Eda decreased the activity of MMP9 and changed progelatinases activity. We conclude that Eda has a perspective to be useful in the treatment of comorbid brain hemorrhage in T2DM due to inhibition of oxidative stress and modulation of gelatinases activity.

Introduction

The study of Type 2 Diabetes Mellitus (T2DM) has been given high priority in recent years, given its high prevalence (around 90% of diabetes mellitus cases worldwide) and the severity of cardiovascular and neurological complications.[1] Micro- and macrovascular disorders due to chronic hyperglycemia are the main causes of high frequency of cardiovascular diseases, stroke and neurological morbidity in Diabetes Mellitus (DM). People with DM have approximately twice risk of non-traumatic Intracerebral Hemorrhage (ICH) and stroke compared to nondiabetic ones, and these patients may have more severe neurological diseases in future.[2] Brain and vascular structural alterations in DM are quite well studied; however, some molecular mechanisms of DM-associated brain disorders remain unclear, although the liter-

ature suggests association of this comorbidity with oxidative stress. In this context, numerous research groups have focused on the study of Reactive Oxygen Species (ROS) sources, their scavenging and signaling, metabolic effects, and the possibilities of antioxidant therapy in T2DM.[3,4]

Hyperglycemia and increased levels of glucose-derived metabolites contribute to the development of diabetic complications partly via increased generation of ROS. Different tissue proteins are effective ROS traps resulting in the Oxidative Modification of Proteins (OMP) with the formation of carbonyl derivatives. Carbonyl groups can be introduced into proteins also by non-oxidative covalent adduction of reactive carbonyl species generated by the oxidation of lipids or carbohydrates. Both direct and indirect carbonylation may affect proteins conformation, activity, and function.[5] Currently, OMP is considered as one of the most harmful and irreversible post-translational protein changes and a key factor in the progression of diabetic complications. Elevated glucose level can also cause accumulation of highly active carbonyl compounds, such as glyoxal/methylglyoxal generating an Advanced Glycation End products (AGEs). Binding of AGEs to their Receptors (RAGE) leads to direct increase of intracellular superoxide production as well as to activation of intracellular signaling pathways NF-κB, p38 MAPK, JNK/SAPK with following increase of superoxide production, creating a "vicious circle" of carbonyl-oxidative stress.[6,7] Furthermore, prolonged hyperglycemia and oxidative stress in DM cause violations of the vascular permeability and blood brain barrier disorders through the Matrix Metalloproteinases (MMP) activity. These zinc-dependent enzymes degrade various protein substrates in Extracellular Matrix (ECM) and take a part in angiogenesis and vascular remodeling, promote endothelial cell proliferation, migration, and differentiation.[8] MMP2 and MMP9, or gelatinases A and B, play a particularly important role in vascular remodeling. These enzymes were proposed as the markers of hemorrhagic stroke, blood-brain barrier disruption, the prognosis of vascular risk in DM as well as targets for the pharmacological therapy.[8-10] Considering the above, we assumed that the study of effects of antioxidant medications on OMP markers and MMP2/MMP9 activity in rats with T2DM and ICH may be useful for choice of rationale therapy in such comorbidity.

Edaravone (Eda) is a drug of interest that was originally developed as a potent free radical scavenger and has been widely used to treat cerebral infarction. 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 demonstrated neuroprotective effects in a number of animal models of disease, including stroke, spinal cord injury, traumatic brain injury, neurodegenerative diseases and brain tumors,[11] but its effects in T2DM are poorly understood. Our working hypothesis for the current study was that Eda may ameliorate brain hemorrhagic transformation in T2DM through impact on the processes of OMP and protein degradation in ECM.

Materials and Methods

All used chemicals were of analytical reagent grade quality produced by Sigma (USA), Bio-Rad Lab (USA), and Reagent (Ukraine). The following drugs were used for the study: edaravone (Eda, "Xavron", Yuria-Pharm, Ukraine), metformin (Met, "Siofor", Berlin-Chemie AG, Germany).

Animals and design of the experiment

The experimental study was performed using albino male Wistar rats that were obtained from Dali-2011 animal facility (Kyiv, Ukraine). Before the experiment, rats were acclimatized for at least 10 days at the animal center of Dnipro State Medical University (DSMU, Ukraine). The animals were housed in groups in rodent cages with standard rat lab chow and filtered tap water *ad libitum* throughout the all-experimental period. Artificial light was provided daily from 08:00 to 20:00, the room temperature and humidity were 22 ± 2 °C and 40–60%, respectively. Nicotinamide-streptozotocin (NA/STZ) model was used for induction of T2DM.[12]

The design of our experiment is presented in the Figure 1, and its detailed description is given below. The inclusion criteria at the start of study were: age of rats 11-12 weeks, body weight of 200–250 g, no signs of physical damage, normal physiological activity and fasting glucose levels (below 6.2 mmol/L).

NA (230 mg/kg, dissolved in saline) and STZ (65 mg/kg, dissolved in 0.1 M citrate buffer, pH 4.5) were administered intraperitoneally (i.p.) to overnight fasted rats (NA was injected 15 min before STZ). Blood glucose concentration was measured 72 hours after NA/STZ injections. Animals with glucose level below 8.3 mmol/L at this time point were excluded from the study.

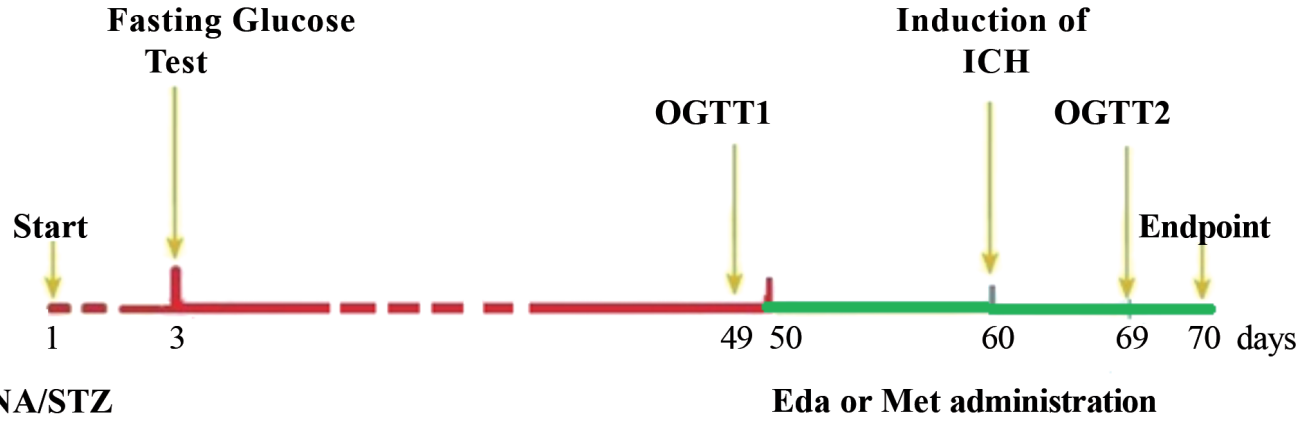

Figure 1. Design of the experiment. The whole duration of the experiment was 70 days and had three key points: i) induction of T2DM by the injection of NA and STZ; ii) induction of ICH; iii) administration of Eda or Met. NA: nicotinamide, STZ: streptozotocin, ICH: intracerebral hemorrhage, Eda: edaravone, Met: metformin, OGTT: oral glucose tolerance test. See the text for details.

Animals were divided randomly into five groups using the results of the Oral Glucose Tolerance Test (OGTT1) conducted on the 49th day after NA/STZ injection: group 1 – the control, saline, 5 mL/kg/day; group 2 – NA/STZ + saline 5 mL/kg/day; group 3 – NA/STZ + ICH + saline 5 mL/kg/day; group 4 – NA/STZ + ICH + Eda 6 mg/kg/day; group 5 – NA/STZ + ICH + Met 250 mg/kg/day. Both drugs, Eda and a classic antidiabetic agent Met, were given for 20 days via intragastral gavage starting from the 50th day of the experiment.

ICH was induced on the 60th day by the following way. Rats were anesthetized by intramuscular injection of tiletamine and zolazepam (Telazol 100, Zoetis Inc., Spain, 30 mg/kg) and xylazine (5 mg/kg, Interchemie, the Netherlands) and then fasten to a stereotaxic frame (1); a burr hole was made and a microsyringe needle was inserted into the striatum by the stereotactic coordinates of 0.2 mm anterior, 2.8-3.0 mm lateral and 5.5 mm ventral to the bregma (2); microinjection of 1 µL sterile saline containing 0.2 IU bacterial collagenase (Type IV-S, Sigma-Aldrich, 1.0 µL of 0.2 IU/µL) was made via a 26G needle for more than 5 min[3]. The needle was left in place for 5 min to prevent backflow, and then it was slowly removed from the brain. The area of craniotomy was sealed with bone wax. The animals of groups 4 and 5 continued to receive Eda and Met after induction of ICH until the end of the experiment.

At the end of experiment (70th day), all animals were sacrificed according to the protocol of the Biomedical Ethics Committee with thiopental sodium (50 mg/kg, i.p.), and blood samples were used for the following studies.

Biochemical analysis

Blood samples were obtained by intracardiac puncture after thoracotomy from the right heart ventricle; sera were separated by centrifugation at 3000 rpm for 15 min and stored at -50 °C for further biochemical analysis.

OGTT2 was performed on the 69th day of the current study. Overnight fasted animals 2 h after drug or saline administration were given 20% glucose solution (2 g/kg) *via* intragastral gavage. Blood glucose levels were measured before (basal glucose level), 30, 60, 90, and 120 min after the administration of glucose solution using blood glucose meter Bionime Rightest GM300 (Switzerland). The area under the glycemic curve was calculated using the trapezoidal rule and expressed as mmol/L×120 min.

Glycated hemoglobin (HbA1c) in whole blood was measured using standard kit ("Reagent", Ukraine) and results were expressed as µmol of fructose/g Hb.

Advanced Oxidation Protein Products (AOPP) were assayed using the modified method of Witko-Sarsat *et al.*[13]AOPP contents were calculated using chloramine standard and expressed as µmol of chloramine-T equivalent per mg of protein (µmol/mg protein).

The level of Carbonylated Proteins (PC) was evaluated by the reaction of protein carbonyl derivatives (phenylhydrazine) in serum with 2,4-dinitro-phenylhydrazine (DNPH).[14] The results were calculated using the molar extinction coefficient 22,000 M^{-1} cm^{-1} for PC370 (neutral) and 16,800 M^{-1} cm^{-1} for PC430 (basic) and expressed as nmol carbonyl/mg of total protein.

AGEs were determined by quantitative fluorescence using the Hoefer DQ 2000 Fluorometer (USA) at fixed wavelengths (excitation/emission – 365 nm/460 nm). The fluorescent emission of serum samples (10-fold diluted in saline) was measured at room temperature and presented in arbitrary units (AU). Quinine hydrochloride (60 mg/L) was used as a standard solution, with the fluorescence level taken as 1000 AU.[15] The final results were expressed as AU per mg of total protein.

Ischemia Modified Albumin (IMA) and IMA/Albumin Ratio (IMAR) were assessed using the albumin cobalt-binding test.[16] Level of albumin was assayed by bromocresol purple dye test. The results were expressed as AU/mg of protein (IMA) or AU/mg of albumin (IMAR).

Gelatin zymography

The activities of MMP2/9 were evaluated using gelatin zymography.[17] Colored markers for electrophoresis (Bio-Rad Lab, USA) and the positive controls of these enzymes (Sigma, USA) were used for identification of latent and mature forms of MMP2/9. The activities of both enzymes were calculated in Arbitrary Units (AU) relative to their activity in the standard sample, where it was taken as 100 AU. The results were expressed as AU per mg of protein.

Statistical analysis

Statistical analysis was performed by GraphPad Prism 9.0 (GraphPad Software, Inc., La Jolla, CA, USA, GPS-2169913-THSG-DF1FF). All data were expressed as mean (x)±SD. The results were analyzed by Shapiro-Wilk test to establish whether the data were normally distributed, followed by a Levene's test to identify variance homogeneity. The data were subsequently tested with a one-way ANOVA and *post hoc* Tukey's Honestly Significant Difference (HSD) to identify any significant differences ($p<0.05$) between experimental groups.

Results

The basal glycemia in diabetic rats of groups 2 and 3 was significantly higher in comparison with animals of group 1, and the mean of basal glucose level in group 3 was slightly lower versus group 2 (Table 1). The results of the OGTT indicated that NA/STZ model of DM corresponds by its parameters to T2DM, which is characterized by impaired glucose tolerance and mild basal hyperglycemia. This is

Table 1. Indexes of glycemic control in the experimental groups.

Group	Basal glucose level (mmol/L)	OGTT, AUC (mmol/L×120 min)	HbA1c (µmol fructose/g Hb)
Group 1 Intact rats (n=8)	5.25±1.28	754.7±212.2	1.31±0.32
Group 2 NA/STZ (n=9)	6.92±1.76[a]	1193±304.8[a]	1.80±0.32[a]
Group 3 NA/STZ+ICH (n=7)	6.59±0.35[a]	1323±206.9[a]	2.02±0.36[a]
Group 4 NA/STZ+ICH+Eda(n=7)	6.90 ±1.40	1124±222.6[a]	1.65±0.23[c]
Group 5 NA/STZ+ICH+Met(n=7)	5.09±1.27[bc]	1006 ±228.0[c]	1.43±0.22[bc]

NA: nicotinamide, STZ: streptozotocin, ICH: intracerebral hemorrhage, Eda: edaravone, Met: metformin, OGTT: oral glucose tolerance test, AUC: area under curve. Data are shown as x±SD. [a]significant difference *vs* group 1, [b]significant difference *vs* group 2, [c]significant difference *vs* group 3, $p\leq0.05$.

supported by our previously published data on changes in the lipid profile and atherogenic index in such experimental model.[18] It should be noted a more significant increase in OGTT and HbA1c in group 3 compared with group 2. Administration of Eda led to decrease of HbA1c level, while Met had a more pronounced effect on both OGTT and HbA1c (Table 1).

As we expected, T2DM was followed by a rise in different OMP-markers (Figure 2). The level of neutral protein carbonyls

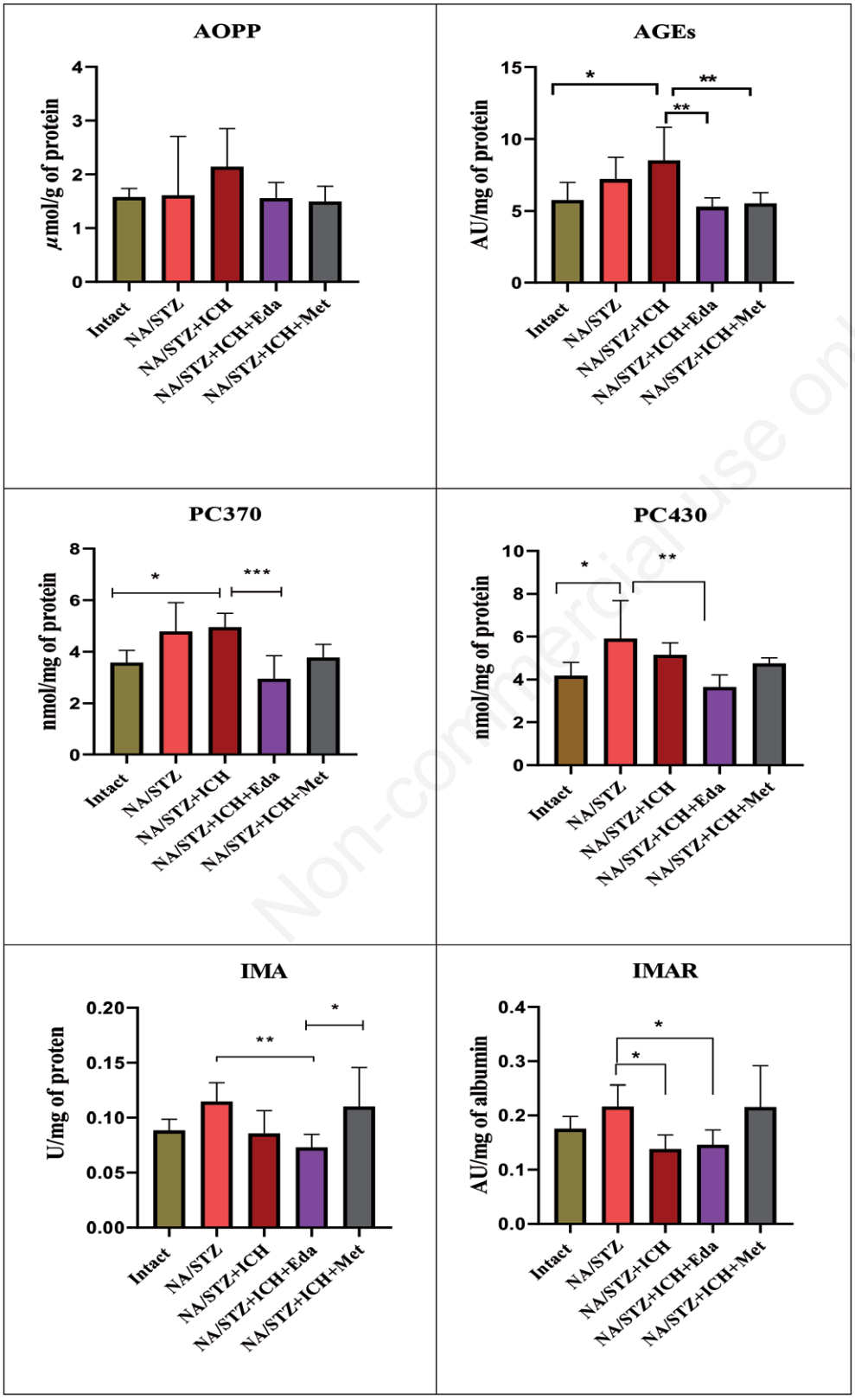

Figure 2. Markers of oxidative status in the experimental groups. NA: nicotinamide, STZ: streptozotocin, ICH: intracerebral hemorrhage, Eda: edaravone, Met: metformin, AOPP: advanced oxidation protein products, AGEs: advanced glycated end products, PC370: neutral protein carbonyls, PC430: basic protein carbonyls, IMA: ischemia modified albumin, IMAR: ratio of ischemia modified albumin to total serum albumin. Statistical significance between groups is indicated as follows: *p<0.05; **p<0.01; ***p<0.001.

PC370 was higher by 27.4%, basic PC430 – by 39.7%, AGEs – by 23.5%, and IMA – by 20.5% compared with intact rats. Interestingly, the mean level of AOPP in T2DM was the same as in the intact rats, although we noted a great variability of this marker in rats with T2DM. Thus, its content in T2DM varied in a range of 0.9-3.4 μmol/mg protein compared to 1.3-1.8 μmol/mg protein for intact animals.

Other changes were observed in group 3 (NA/STZ+ICH), where AOPP, PC370 and AGEs had a tendency to an increase, and PC430 was decreased versus group 2. As for IMA, its level in this group was equivalent to those in intact rats.

Administration of Eda or Met led to decrease in levels of all the above-mentioned markers with the most significant changes in PC and AGEs in Eda-treated animals. At that time, Eda had a mild effect on basal glucose level and glucose tolerance in comparison with Met (Table 1).

The second part of our research was devoted to studying the effect of Eda on the activity of matrix metalloproteinases MMP2 and MMP9 with an emphasis on the ratio of latent and mature forms of these enzymes. There are only a few reports on the effectiveness of Eda in traumatic brain injuries and cerebral ischemia, which discuss the effect of this drug only on the expression of MMP9,[11,19] while its possible effect on MMP2, which has similar substrate specificity, is not taken into account. Our previous experience indicates the importance of assessing the ratio of various gelatinases activity in cardiovascular pathology,[15] based on which we decided to carry out similar calculations in the current study.

The results of our study demonstrated the difference in changes of MMP2 and MMP9 activity in NA/STZ rats at 21st day after ICH: proMMP9 was decreased, while the mature forms of both gelatinases were slightly increased (Figure 3). The calculation of the proMMP9/proMMP2 ratio showed a significant ($p<0.05$)

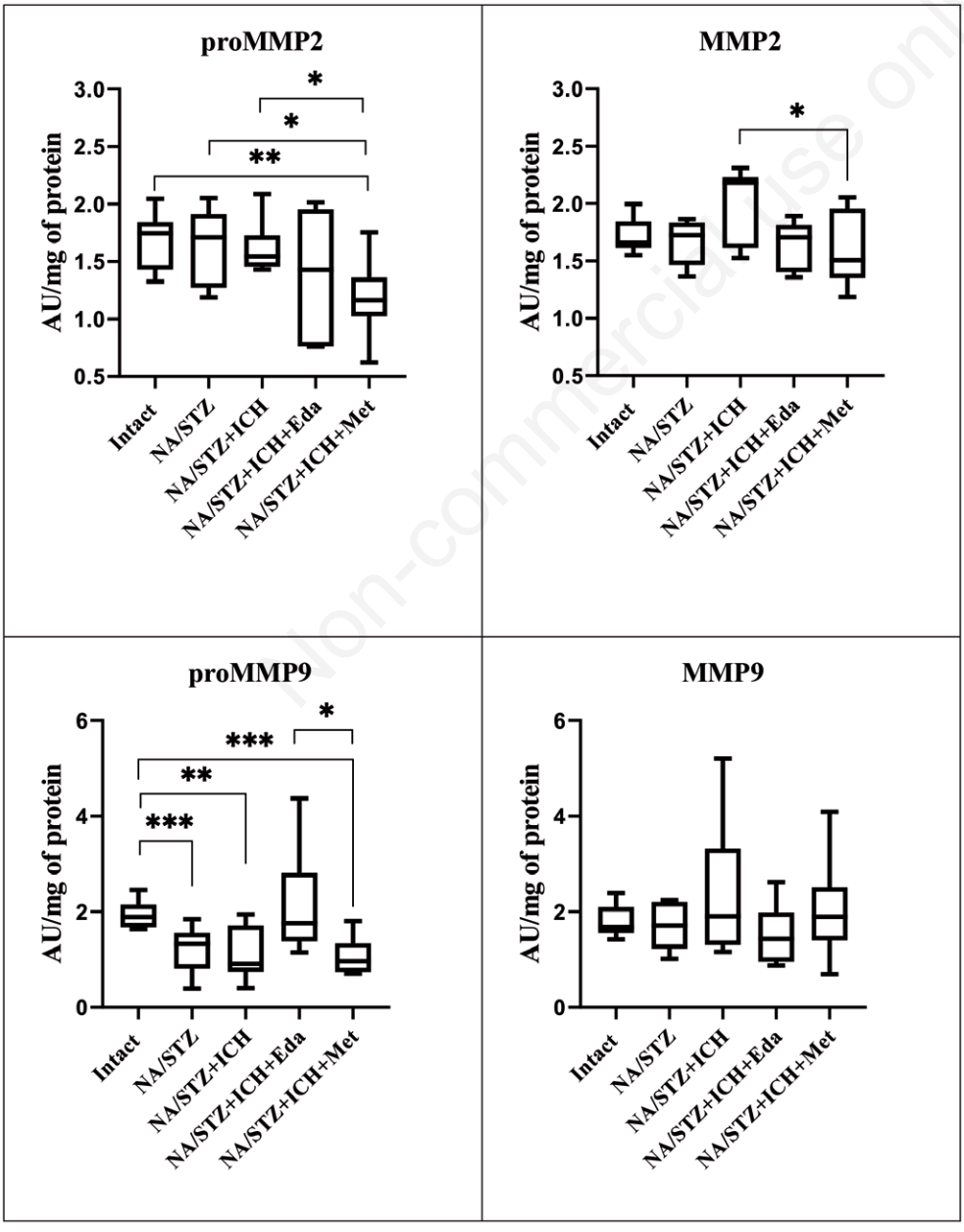

Figure 3. Activity of matrix metalloproteinases in serum in the experimental groups. NA: nicotinamide, STZ: streptozotocin, ICH: intracerebral hemorrhage, Eda: edaravone, Met: metformin, MMP: matrix metalloproteinase. Statistical significance between groups is indicated as follows: *p<0.05; **p<0.01; ***p<0.001.

decrease of this ratio from 1.21±0.11 in intact rats to 0.79±0.18 in group 3 (NA/STZ+ICH). The ratio MMP9/MMP2 changed in the opposite way, increasing from 1.04±0.06 in group 1 to 1.22±0.23 in group 3.

According to our results, Eda reduces the activity of all studied MMPs in comparison with group NA/STZ+ICH, with the exception of proMMP9, the level of which rises above its normal values (Figure 3). Significant reduction in proMMP2 and MMP2 activity was observed after administration of Met. Correlation analysis revealed a strong association between the latent and mature forms of both gelatinases in Eda treatment: the Pearson coefficient was 0.874 (p=0.023) for the proMMP2/MMP2 pair and 0.922 (p=0.009) for the proMMP9/MMP9 pair in group NA/STZ+ICH+Eda. Calculation of this coefficient for the pair MMP2/MMP9 demonstrated a lack of correlation between these enzymes after Eda administration (r=0.32, p=0.536). In contrast, there was a strong positive correlation for the pair MMP2/MMP9 (r=0.822, p=0.013) in Met treated group.

Discussion

The results of our study indicated that the current NA/STZ model of T2DM is characterized by impaired glucose tolerance and mild basal hyperglycemia with a more significant increase in OGTT and HbA1c in group 3 (NA/STZ + ICH) compared with group 2 (NA/STZ). Additionally, this experimental model was followed by a rise in some OMP-markers (PC370, PC430, AGEs, and IMA). Although, there was no significant difference in the level of AOPP between group 2 and group 1 (intact rats), and greater variability of this marker was noted in rats with T2DM. These results may be explained by the differences in ways of formation and definition of various OMP markers. It is known that the formation of PC and AGEs includes the introduction of additional carbonyl groups into proteins due to the oxidation of side chains of some amino acids or their interaction with ROS as well as due to non-oxidative reactions with oxidized lipids containing carbonyl groups. Recently, Nair *et al.*[6] identified 13 consistently expressed proteins with carbonylated residues of Lys, Val, Ile, Cys, Thr, and Asp in monocyte cells under hyperglycemic stress. The glycolytic enzymes (fructose-bisphosphate aldolase, α-enolase), as well as carbonic anhydrase (which is associated with T2DM and insulin resistance) were detected among carbonylated proteins. The altered functionality and ability to proteolytic degradation of above mentioned carbonylated proteins in hyperglycemia and their association with inflammation and complications of DM were demonstrated in the study.[6] In contrast to carbonylation, the major pathway of AOPP formation is the myeloperoxidase-mediated reaction of chlorinated oxidants and proteins. A significant increase of AOPP in serum and a decrease of this marker in leukocytes of rats with streptozotocin-induced DM was shown.[20] Authors supposed that organic chloramines can be slowly hydrolyzed into aldehydes, thus increasing the PC pool of neutral and basic nature in blood plasma under DM. Our results on changes in PC and AOPP in the blood of NA/STZ rats fully agree with these data.

The level of IMA in group 3 was also characterized by minimal changes as compared to intact rats. These results can be explained by the conditions of our experiment and the peculiarities of IMA metabolism. The previous research of this marker has demonstrated its increase within 6-10 min after acute myocardial ischemia with the following recovery to the initial values within 6-12 hours.[21] Decrease of this marker after ICH may be associated also with changes in the level of endogenous lactate after collagenase-induced ICH, development of brain edema and inflammation, and activation of glycolysis in these animals. However, the relationship between IMA and lactate levels remains a subject for discussion and requires additional research.[22]

In this study, administration of Eda or Met led to a decrease in levels of markers of oxidative protein modification. Our results are in accordance with recent studies, which showed that Eda modulates endothelial function and elicited its protective effect via suppression of ROS release as well as by inhibition of AGEs/RAGE/oxidative stress and decrease of OMP.[6,11,23] As for Met, this first-line drug for T2DM treatment has not only a hypoglycemic effect but also demonstrates a positive effect on posttraumatic neurological complications via its ability to reduce brain edema and inhibit neuronal necroptosis after ICH. Recently, it was shown that Met can exhibit these effects via both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms.[24,25] Based on the results of this study, we may suggest that a decrease of OMP by Eda and Met is an additional mechanism of their therapeutic effects in T2DM.

Next, we studied the activity of matrix metalloproteinases MMP2 and MMP9 (their both latent and mature forms). Our results demonstrated an opposing tendency in changes of MMP2 and MMP9 activity in diabetic rats with ICH: a decrease in proMMP9, and a small increase in the mature forms of both gelatinases. As a consequence, it was found that proMMP9/proMMP2 ratio was significantly less in group 3 as compared to intact rats. Analysis of the literature showed that the differences described above might be associated with temporal changes in serum MMP9 and MMP2 activities depending on the acute and subacute phases of ICH. According to Castellazzi *et al.*,[26] serum concentration of MMP9 in patients with spontaneous ICH was increased at 48 h and peaked at 7 days, and the mean of MMP2 progressively declined during 2 to 7 days. Moreover, the authors found a positive correlation between perihematomal edema volume and levels of MMP2 at 24 h, and with MMP9 activity at 48 h in these patients.[26]

Analysis of relations between gelatinases' activity and the volume of cerebral edema in different cohorts of patients with ICH, which was presented in the review of Lattanzi *et al.*, indicates significant differences depending on the output of the disease.[27] The authors believe that the effect of the MMP-mediated response in the course of ICH may vary according to the MMP type. Some studies imply that MMP9 is involved in the acute phase, whereas MMP2 acts more in the chronic phase.[27] The findings of our experiment let us suppose that changes in proMMP9/proMMP2 ratio in serum at a more prolonged period after ICH may reflect the activity of inflammation in the brain. Thus, the time profile of gelatinases expression and the ratio of their forms may be pivotal when thinking about the therapeutic aspects of studied drugs.

The difference in gelatinases activity after Eda and Met administration found in our study may be caused by the particularities of MMPs regulation. As was shown by Miyamoto *et al.*, Eda can directly inhibit MMP-9 expression in oligodendrocyte precursor cells and reduce blood-brain barrier damage in cerebral white matter under prolonged cerebral hypoperfusion in mice.[28] Met may impact on MMP9 expression through regulation of hsa-miR-21-5p.[29] Recently, it was demonstrated that MMP9 and hsa-miR-21-5p were downregulated and upregulated respectively in patients with T2DM and diabetic nephropathy and the patients showed downregulation of hsa-miR-21-5p and upregulation of MMP9 after metformin treatment.[29] The effects of Met on gelatinase activity in rats with T2DM+ICH in our study are not entirely consistent with these data. Targeted analysis of the expression of these enzymes under experimental conditions is required.

The beneficial effect of Eda on serum MMP9 was demonstrated in the clinical analysis of 160 patients with basal ganglia hemorrhage. In this study, a significantly decreased MMP9 level in the group of patients after Eda treatment in comparison with the control group was found. Further analysis revealed that these changes were correlated (r=0.491) with the National Institute of Health Stroke Scale (NIHSS) and Glasgow Coma Scale scores.[30] Gelatinases inhibition represents a potentially effective target for neuroprotection in ICH. However, these enzymes have a dual role, because they not only disturb the components of ECM but take a part in angiogenesis and vascular remodeling.[8,9] Taking this into account, the inhibition of pathologic processes in the brain must be balanced against the preservation of neuroprotective angiogenesis. So, further studies are necessary to delineate mechanisms, dose, and time of Eda use in T2DM patients with neurological comorbidity.

Although this study allowed us to clarify the biochemical effects of Eda in T2DM complicated by ICH, nevertheless it has some limitations. First of all, it concerns the issue of equivalency of the chosen animal NA/STZ model to T2DM in humans. The best model of DM should have some major criteria such as the following: the model should have all major signs of DM with other possible complications that can also be found in diabetic humans, the model should be sensitive to antidiabetic drugs, and the model needs to be suitable to study the pathogenesis of the disease as well as for routine pharmacological screening of antidiabetic drugs. Human T2DM is known to be accompanied by increased lipogenesis and obesity on the background of insulin resistance. However, we did not find significant weight gain or morphological signs of obesity in experimental animals. Our previously published results[18] nonetheless demonstrated a significant increase in the level of serum triglycerides that indicates the activation of lipogenesis.

Another issue that must be taken into account is the conditions of the experiment. According to the study design, the daily administration of Eda began 10 days before the induction of ICH and continued for 20 days until the end of the experiment. Such scheme does not allow to differentiate the preventive and therapeutic effects of the drug. Apparently, in the future, it will be necessary to evaluate the preventive effect of Eda by changing the experimental scheme in an appropriate way and to analyze the OMP and MMP in the dynamics of the inflammatory response development after induction of ICH. Moreover, the evaluation of proteolytic balance in blood, including the analysis of the expression of MMPs and their inhibitors, as well as the activity of the fibrinolytic system, is required for the assessment of blood-brain barrier functionality.

Summing up, the following main findings of this study should be noted. Eda reduces OMP in rats with experimental T2DM complicated by collagenase-induced ICH and modulates the activity of MMP2 and MMP9. The results of the study provide a basis for understanding the relationship between OMP and proteolysis in vascular and neurological disorders associated with T2DM. Obtained results may be potentially interesting to design the strategies for the diagnosis and control of stroke treatment in patients with DM.

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