UDC 616.831-005.1-002.1:616.379-008.64]-092.9-07-085 https://doi.org/10.26641/2307-0404.2021.4.248055

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THE IMPACT OF PERINDOPRIL AND METFORMIN ON THE MARKERS OF ENDOTHELIAL DYSFUNCTION IN RATS WITH ACUTE INTRACEREBRAL HEMORRHAGE AND TYPE 2 DIABETES MELLITUS

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Цитування: Медичні перспективи. 2021. Т. 26, № 4. С. 15-22
Cited: Medicni perspektivi. 2021;26(4):15-22

Key words: *type 2 diabetes mellitus, endothelial dysfunction, intracerebral hemorrhage, perindopril, metformin*
Ключові слова: *цукровий діабет 2 типу, ендотеліальна дисфункція, інтрацеребральний крововилив, периндоприл, метформін*
Ключевые слова: *сахарный диабет 2 типа, эндотелиальная дисфункция, внутримозговое кровоизлияние, периндоприл, метформин*

Abstract. The impact of perindopril and metformin on the markers of endothelial dysfunction in rats with acute intracerebral hemorrhage and type 2 diabetes mellitus. Zhyliuk V.I., Lievykh A.E., Shevtsova A.I., Tkachenko V.A, Kharchenko Yu.V. *This comparative research is aimed to study the effect of perindopril and metformin on the levels of biochemical markers of endothelial dysfunction in rats with type 2 diabetes mellitus (T2DM) complicated by a brain hemorrhage. The study was carried out on 30 white male Wistar rats. T2DM was simulated by a single intraperitoneal injection of nicotinamide and streptozotocin (NA/STZ). Intracerebral hemorrhage (ICH) was induced by microinjection of 1 μL of bacterial collagenase 0.2 IU/μL into the striatum on the 60th day of the experiment. Animals were randomized into 5 groups: A – negative control (intact, n=6); B – positive control 1 (NA/STZ, n=6); C – positive control 2 (NA/STZ+ICH, n=6); D – perindopril ("Prestarium", 2 mg/kg+NA/STZ+ICH, n=6); E – metformin ("Siofor", 250 mg/kg+NA/STZ+ICH, n=6). The studied drugs were administered intragastrically for 20 days, starting from the 50th day after the induction of T2DM. Endothelial function was assessed by the content of homocysteine (Hcy), advanced glycation end products (AGEs), endothelin-1 (ET-1), and von Willebrand factor (vWF) in blood serum. It was found that long-term separate T2DM is accompanied by hyperhomocysteinemia, as well as an increase in AGEs, ET-1, and vWF levels, indicating dysregulation of the hemostasis system and vascular tone. It should be noted that brain hemorrhage in T2DM can enhance these manifestations, although the obtained differences were characterized only by a persistent trend. At the same time, the effect of perindopril was limited only by a significant decrease in AGEs levels by 31.2% ($p<0.05$). In turn, the action of metformin was characterized by a positive glycemic control, as well as an effect on the state of the vascular endothelium, namely, a significant decrease in AGEs, ET-1 and vWF levels by 37.6% ($p<0.05$); 5.5% ($p<0.05$) and 9.5% ($p<0.05$), respectively. It was also found that the endotheliotropic properties of the studied drugs were not associated with an effect on homocysteine levels. Thus, metformin in conditions of diabetes mellitus complicated by acute intracerebral hemorrhage has advantages over perindopril in relation to endothelial dysfunction.*

Реферат. Влияние периндоприла и метформина на маркеры дисфункции эндотелия у крыс с острым интрацеребральным кровоизлиянием при сахарном диабете 2 типа. Жилюк В.И., Левых А.Э., Шевцова А.И., Ткаченко В.А., Харченко Ю.В. *Целью данного сравнительного исследования было изучение влияния периндоприла и метформина на уровни биохимических маркеров эндотелиальной дисфункции у крыс при сахарном диабете 2 типа (СД2), осложненном геморрагическим поражением головного мозга. Эксперимент проведен на 30 белых крысах-самцах линии Вистар. Моделирование СД2 осуществляли однократным внутрибрюшинным введением никотинамида и стептозотоцина (НА/СТЗ). Внутримозговое кровоизлияние (ВМК) формировалось путем микроинъекции 1 мкл бактериальной коллагеназы 0,2 МЕ/мкл в область стриатума на 60 день эксперимента. Животные были рандомизированы на 5 групп: А – негативный контроль (интактные, n=6); В – позитивный контроль 1 (НА/СТЗ, n=6); С – позитивный контроль 2 (НА/СТЗ+ВМК, n=6); D – периндоприл ("Престариум", 2 мг/кг+НА/СТЗ+ВМК, n=6); Е – метформин*

("Сиофор", 250 мг/кг+НА/СТЗ+ВМК, n=6). Исследуемые препараты вводили внутрижелудочно в течение 20 дней, начиная с 50 дня от индукции СД2. Функция эндотелия оценивалась по содержанию гомоцистеина (Гц), конечных продуктов гликирования (КПГ), эндотелина-1 (ЕТ-1) и фактора фон Виллебранда (фВ) в сыворотке крови. Установлено, что длительное течение изолированного СД2 сопровождается гипергомоцистеинемией, а также повышением уровней КПГ, ЕТ-1 и фВ, свидетельствующих о нарушении регуляции системы гемостаза и сосудистого тонуса. Следует отметить, что геморрагическое поражение головного мозга при СД2 может усиливать указанные проявления, хотя полученные отличия носили лишь характер стойкой тенденции. При этом влияние периндоприла было ограничено только значимым на 31,2% (p<0,05) снижением уровней КПГ. В свою очередь, действие метформина характеризовалось как положительным гликемическим контролем, так и влиянием на состояние эндотелия сосудов, а именно – достоверным уменьшением уровней КПГ, ЕТ-1 и фВ на 37,6% (p<0,05); 5,5% (p<0,05) и 9,5% (p<0,05) соответственно. Также установлено, что эндотелиотропные свойства исследуемых препаратов не были связаны с влиянием на уровни гомоцистеина. Таким образом, метформин в условиях сахарного диабета, осложненного острым интрацеребральным кровоизлиянием, имеет преимущества перед периндоприлом в отношении эндотелиальной дисфункции.

Type 2 diabetes mellitus (T2DM) belongs to the group of chronic metabolic diseases and is caused by insulin resistance or deficiency, resulting in increased blood glucose levels. This form of diabetes is the most common, as it accounts for 90-95% of patients with diabetes mellitus (DM). Poorly controlled diabetes mellitus is associated with an increase in morbidity and mortality, and the main cause of death is cardiovascular disease, including atherosclerosis and hypertension [14]. Insulin resistance in diabetes mellitus is associated with many risk factors that usually precede the development of hyperglycemia. These typically include obesity, dyslipidemia, which is characterized by high triglycerides, high blood pressure, oxidative stress, and endothelial dysfunction (ED) [14]. Endothelial cells, like their major products, nitric oxide (NO) and prostacyclin play a key role in the regulation of vascular homeostasis. Diabetes-induced ED is a critical and initiating factor in the development of diabetic vascular complications. It is characterized by reduced bioavailability of NO, reduced synthesis of prostacyclin and endothelial hyperpolarization factor (EHF) and increased production or action of endothelial vasoconstrictors (endothelin-1, von Willebrand factor, etc.) [13]. In addition, hypertension and dyslipidemia lead to an imbalance in angiotensin II – bradykinin homeostasis, which is an additional factor in the development of ED and changes in blood vessels that contribute to atherosclerosis and thrombosis [10].

Elevated levels of glucose and / or lipids that accompany diabetes and obesity cause hyperproduction of highly active carbonyl compounds and reactive oxygen species (ROS) and, as a result, initiate the development of "carbonyl" and "oxidative" stresses. In particular, glycotoxins and lipotoxins are able to react quickly and damage various cellular and extracellular molecules, forming the advanced glycation end products (AGEP) [6, 15]. Binding of AGEP to their receptors (RAGEP) increases the intracellular enzymatic production of superoxide, which, in turn, not only has direct harmful effects, but also activates various intracellular signaling pathways, such as NF-κB, p38 MAPK, JNK/SAPK, hexosamine pathway, protein kinase C, AGEP /RAGEP, TNFα and sorbitol synthesis, which further enhances superoxide production and creates a "endless circle effect". In addition, AGEP can accumulate in the vascular wall, which reduces the activity of nitric oxide (NO) and the expression of its synthase (eNOS) and increase the expression of ET-1, which underlie the development of ED [3, 6]. Today, protein carbonylation is one of the most harmful and irreversible modifications and is considered a key factor in the progression of diabetes and associated complications [9]. Hyperhomocysteinemia is also an important factor in ED, as it can reduce the synthesis and bioavailability of NO and EHF, promote the production of vasoconstrictive prostanoids and activation of AT1-receptors of angiotensin II (ATII), as well as ROS generation by phosphorylation of NADPH/oxide activity of angiotensin-converting enzyme (ACE) and the formation of ATII, which also activates NADPH oxidase [14].

Although diabetes is an independent risk factor, primarily for ischemic stroke, it is also associated with an increased risk of intracerebral hemorrhage (IH), which is directly related to the duration of diabetes. It is likely that there is a J-like relationship between IH and glycosylated hemoglobin (HbA1c) levels, suggesting that both poor control and extremely intensive control of diabetes lead to an increased risk of IH [4].

Perindopril belongs to the group of ACE inhibitors (ACEI). It is nown known that ACE inhibitors are able to improve endothelial function by increasing NO production both directly and indirectly. This effect is associated with the prevention of ATII synthesis, which reduces NO production, and the inhibition of bradykinin degradation, which

stimulates local NO release. In the ACEI class, perindopril has the highest selectivity for the site of bradykinin binding compared to other members of this group [2]. The combination of lipophilic profile, high tissue affinity and high selectivity of bradykinin / angiotensin I provide perindopril complete and long-term inhibition of bradykinin degradation and may have a greater physiological effect compared to other ACE inhibitors [2]. Under conditions of DM and disorders of nitratergic regulation of vascular tone, the increase in the proportion of the bradykinin system may play a key compensatory role.

Metformin belongs to the group of biguanides and is the main drug for the treatment of T2DM. Metformin lowers blood glucose levels, mainly by increasing the sensitivity of the liver, muscle, adipose tissue and other tissues to glucose, as well as reducing insulin resistance. The positive fact is that metformin does not cause hypoglycemia, so it is also called "antihyperglycemic agent" [6]. The metabolic effects of metformin in diabetes mellitus may also provide it with endothelioprotective properties [6].

The aim of this comparative study was to examine the effect of perindopril and metformin on the level of biochemical markers of endothelial dysfunction in the blood of rats with acute intracerebral hemorrhage on the background of streptozotocin-nicotinamide-induced diabetes.

MATERIALS AND METHODS OF RESEARCH

The study was conducted at the Department of Pharmacology and Clinical Pharmacology, Dnipro State Medical University (Dnipro). All procedures (anesthesia, administration of drugs, withdrawal of animals from the experiment, etc.) fully complied with the principles of Directive N 2010/63/EC on the protection of animals used for scientific purposes (2010), the Law of Ukraine "On protection of animals from ill-treatment" and the conclusion of the commission on biomedical ethics of DSMU (protocol No. 8 of 17.12.2019).

Type 2 diabetes mellitus was induced by a single intraperitoneal administration of nicotinamide (NA, 230 mg/kg, Sigma-Aldrich, USA) and streptozotocin (STZ, 65 mg/kg, AdooqBioscience, USA) to rats on an empty stomach in citrate buffer (pH=4.5, 0.1 M) [8]. Blood glucose levels were measured 72 hours after diabetes induction. The study included animals with a blood glucose content of not less than 8.3 mmol/l [7].

Intracerebral hemorrhage (IH) was induced by microinjection of 1 µl dissolved in sterile saline of 0.2 IU of bacterial collagenase (type IV-S, 1 µl 0.2 IU/µl, "Sigma-Aldrich", USA) on day 60 of the study by stereotactic coordinates: anterior-posterior – 0.2 mm in front of the bregma, mediolateral – 2.8-3.0 mm on the right side of the bregma, depth – 5.5 mm which corresponded to the striatum area [5].

According to the results of the glucose tolerance test, the animals were divided into five groups: A – negative control [intact, saline, 5 ml/kg/day, per os] (n=6); B – positive control 1 [NA/STZ+saline, 5 ml/kg/day] (n=6); C – positive control 2 [NA/STZ+IH+saline, 5 ml/kg/day] (n=6); D – animals that received perindopril (Prestarium, Servier, Ireland) at a dose of 2 mg/kg/day [NA/STZ+IH+Per] (n=6); E – animals that received metformin (Siofor®, Berlin Chemie AG, Germany) at a dose of 250 mg/kg/day [TA/STZ+IP+Met] (n=6).

From the day 50 of daily induction of T2DM, for 20 days animals received test preparations or saline intragastrically using a probe.

Fasting glucose levels were measured with a Bionime Rightest GM300 meter (Bionime Corporation, Switzerland) in blood samples obtained from the tail vein. The oral glucose tolerance test was performed twice – on day 49 and 69 of this study. Areas under glycemic curves (blood glucose area under the curve, AUCglu) were calculated using GraphPad Prism 8.0 software.

On day 70 of the experiment, all animals were removed from the experiment by administration of sodium thiopental (50 mg/kg, intraperitoneally). Venous blood samples in a volume of 5 ml were obtained by puncture of the right ventricle of the heart. The content of homocysteine (Hc) in the serum was measured by enzymatic method using a standard test kit "Homocysteine, enzymatic cycling" ("DIALAB® GmbH" Wr. Neudorf, Austria) and biochemical analyzer HTI BioChem SA (High Technology Inc., USA). Levels of glycosylated hemoglobin (HbA1c) in whole blood were determined spectrophotometrically using a standard test kit ("PJSC Reagent", Ukraine) and expressed in µmol of fructose/g Hb [8].

The content of advanced glycation end products (AGEP) was measured by the fluorescence method [12], using a fluorometer Hoefer DQ 2000 (USA) with a fixed wavelength (excitation / radiation =365 nm/460 nm). The obtained data were presented in conventional units (CU) per mg of protein (CU/mg of protein).

Serum levels of endothelin-1 (ET-1) and von Willebrand factor (fV) were determined by enzyme-linked immunosorbent assay using a semi-automatic ELISA analyzer RT 2100 (Rayto, China), using test kits "Rat Endothelin-1 (ET-1) ELISA Kit, Catalog # MBS3808173" and "Rat Von Willebrand Factor

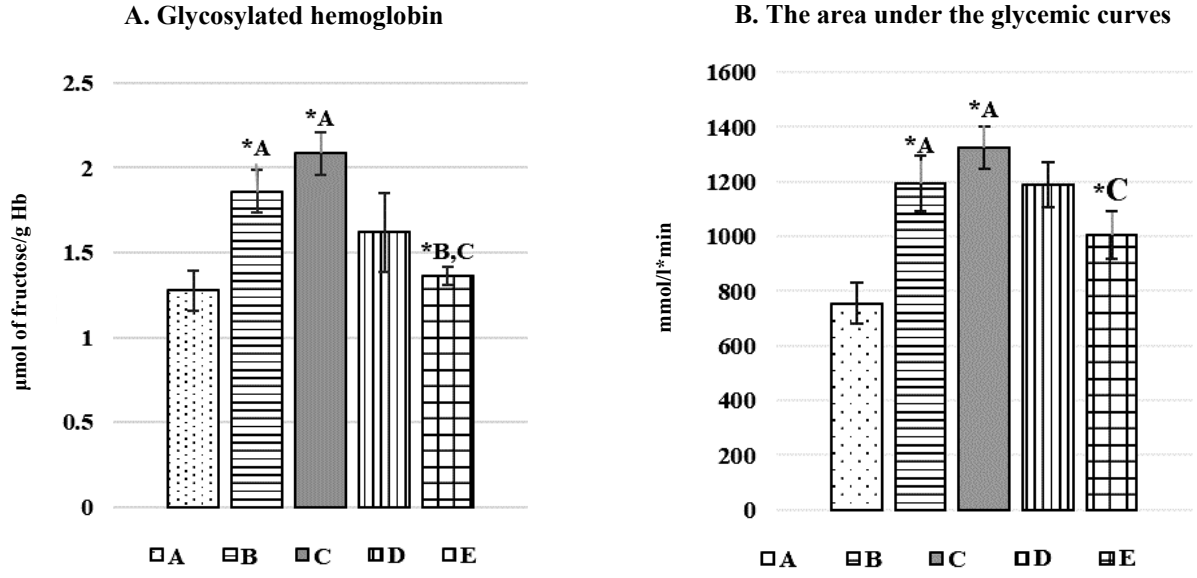
(vWF) ELISA Kit, Catalog # MBS775527" (MyBioSourse, Inc., San Diego, CA, USA).

The data obtained in the experiment were processed by statistical methods using the licensed program GraphPad Prism 9.0 (GraphPad Software, Inc., La Jolla, CA, USA, GPS-2169913-THSG-DF1FF). The reliability of intergroup differences was established using the parametric Student's t-test or one-way analysis of variance ANOVA and the Mann-Whitney or Kruskal-Wallis test for abnormal distribution [1]. The level of statistical significance of the obtained results was $p<0.05$.

RESULTS AND DISCUSSION

The results of the study indicate that the reproduction of T2DM (group B, NA/STZ) in rats was characterized by an increase in HbA1c content by 45.7%, $p<0.05$ relative to group A (intact). At the same time, in rats with T2DM and IH (group C, NA/STZ+IH) values of this indicator were by 63.4% higher than in group A, $p<0.05$ (Fig. 1). Metformin, but not perindopril, statistically significantly reduced HbA1c levels as compared with group B by 26.7%, $p<0.05$, and with group C – by 34.6%, $p<0.05$. The values of HbA1c in group D (NA/STZ+IH+Per) were characterized only by a clear tendency to decrease, especially in relation to animals of group C, but had no statistical significance (Fig. 1A).

Presented in Fig. 1A analysis of glycemic curves (AUCglu) shows that the course of T2DM (group B) led to the development of glucose tolerance, as evidenced by an increase in the area under the curve by 45.9%, $p<0.05$. Moreover, the simulation of IH (group C) did not significantly change the value in this test, and its value was by 1.62 times, higher $p<0.05$ than the data of group A. It was metformin, not perindopril, that statistically significantly by 26.6%, $p<0.05$, decreased the AUC area relative to group C, but not group B.

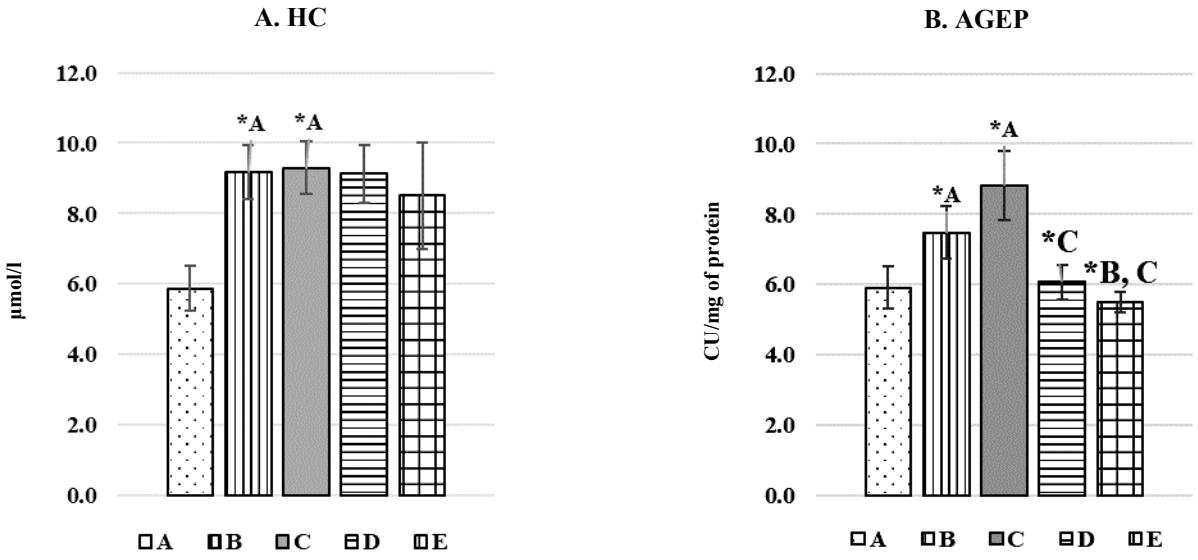


Notes: A – negative control (intact); B – positive control 1 (NA/STZ); C – positive control 2 (NA/STZ+IH); D – perindopril (NA/STZ+IH+Per); E-metformin (NA/STZ+IH+Met); * – $p<0.05$ – statistical significance.

Fig. 1. Content of glycosylated hemoglobin (HbA1c) and the area under the glycemic curves (AUCglu) in the blood of rats with T2DM and IH in terms of administration of perindopril and metformin

Homocystein (HC) levels, as a predictor of ED and atherogenesis in T2DM were also characterized by a significant increase, but intracerebral hemorrhage did not significantly change its severity (Fig. 2A). At the same time, experimental pharmacotherapy did not affect any of the drugs used at the HC level in rats.

It should be noted that the long course of T2DM and IH reproduction under these conditions was accompanied by a statistically significant increase of serum levels of AGEP (Fig. 2B) by 1.46 times, $p<0.05$, and by 1.62 times, $p<0.05$, respectively. At the same time, both perindopril and metformin reduced the content of these markers of carbonyl stress by 31.2%, $p<0.05$, and 37.6%, $p<0.05$, respectively. Moreover, in contrast to perindopril, the effect of metformin was manifested relative to group B (Fig. 2B).



Notes: A – negative control (intact); B – positive control 1 (NA/STZ); C – positive control 2 (NA/STZ+IH); D – perindopril (NA/STZ+IH+Per); E – metformin (NA/STZ+IH+Met); * – p<0,05 – statistical significance.

Fig. 2. Content of homocysteine (HC) and advanced glycation end products (AGEP) in the serum of rats with T2DM and IH in terms of administration of perindopril and metformin

During the experiment, it was determined that the levels of ET-1 increased the most in animals of group C (Fig. 3A). Moreover, both research means equally reduced its content under these conditions, but only the introduction of metformin provided statistically significant changes in this indicator for animals of group C (Fig. 3A).

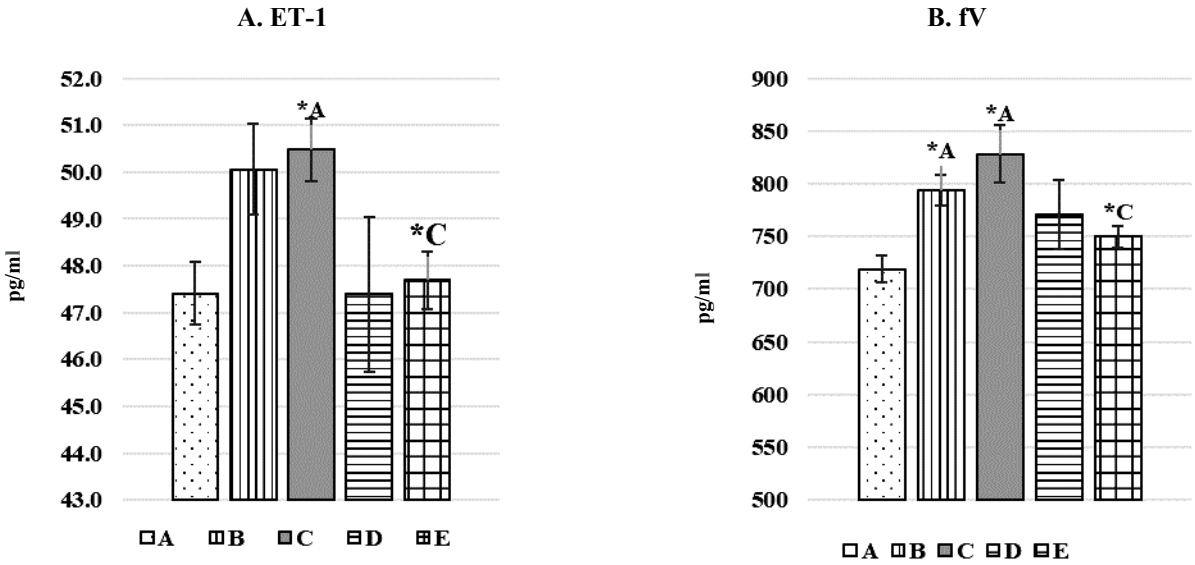
Similar changes were recorded for von Willebrand factor levels (Fig. 3B). It was found that the course of T2DM, both isolated and complicated by intracerebral hemorrhage, led to an elevation of fV in serum by 10.4%, p<0.05, and 15.2%, p<0.05, respectively. At the same time, only experimental metformin therapy contributed to a significant reduction in fV by 9.5%, p<0.05, as compared with group C (Fig. 3B).

Thus, the long-term course of isolated T2DM is characterized by hyperhomocysteinemia, as well as increased serum levels of end products of glycation, endothelin-1 and von Willebrand factor, which may indicate the initiation of oxidative and carbonyl stress, as well as disorders of hemostasis and vascular tone regulation. These processes are key in the development of endothelial dysfunction, atherogenesis and thrombotic complications in T2DM. It should be noted that hemorrhagic brain damage in T2DM may exacerbate these processes, although in our studies statistical significance between the two groups of positive control was not observed, and the differences were stable.

Interestingly, the therapeutic effects of the studied drugs were in no way related to the effect on homocysteine levels, and the effect of perindopril was limited to a significant reduction in AGEP levels.

At the same time, the pharmacological properties of metformin in conditions of diabetes mellitus complicated by IH were characterized by both positive glycemic control and effects on vascular endothelium, namely the reduction of carbonyl stress and endothelial dysfunction – ET-1 and fV. The endothelial effects of metformin are probably mediated by many mechanisms. It is known that this agent is able to increase the concentration of hydrogen sulfide gas transmitter (H2S) in tissues, which functions due to sulfhydration of potassium channels and acts as an EHF [6]. Metformin is also able to reduce the effects of endothelial dysfunction induced by glucose fluctuations by improving the intracellular assembly of eNOS and inhibition of NADPH oxidase [11].

Thus, it can be assumed that the use of metformin in diabetes, in particular complicated by hemorrhagic brain damage, has advantages over perindopril as for the manifestations of diabetes-associated endothelial dysfunction.



Notes: A – negative control (intact); B – positive control 1 (NA/STZ); C – positive control 2 (NA / STZ + IH); D – perindopril (NA/STZ+IH+Per); E-metformin (NA / STZ + IH + Met); * – p <0.05 - statistical significance.

Fig. 3. Levels of endothelin 1 (ET-1) and von Willebrand factor (fV) in the serum of rats with T2DM and IH in terms of administration of perindopril and metformin

CONCLUSIONS

1. Hemorrhagic brain damage in type 2 diabetes mellitus shows a tendency to increase the severity of endothelial dysfunction caused by streptorotozotocin-nicotinamide-induced diabetes mellitus in rats.
2. In experimental type 2 diabetes mellitus complicated by intracerebral hemorrhage, perindopril reduces serum advanced glycation end products but does not affect glycemia and endothelin-1 and von Willebrand factor levels.
3. Metformin inhibits carbonyl stress and improves both glycemic status and endothelial function in diabetic rats with Type 2 diabetes and hemorrhagic brain damage.
4. Metformin and perindopril do not affect the manifestations of hyperhomocysteinemia caused by diabetes mellitus.

Conflict of interest. The authors declare no conflict of interest.

The research was supported by the Ministry of Health of Ukraine, Order No. 509 of 24.02.2020 within the RW: "Study of endothelium-platelet relations in the mechanisms of organoprotective action of drugs in conditions of hyperglycemia and insulin resistance" (0120u101502, 2020-2022).

REFERENCES

1. Antomonov MYu. [Mathematical processing and analysis of medical and biological data]. Kyiv: Malyi druk; 2006. p. 558. Russian.
2. Ancion A, Tridetti J, Nguyen Trung ML, Oury C, Lancellotti P. A Review of the Role of Bradykinin and Nitric Oxide in the Cardioprotective Action of Angiotensin-Converting Enzyme Inhibitors: Focus on Perindopril. Cardiol Ther. 2019;8(2):179-91. doi: https://doi.org/10.1007/s40119-019-00150-w
3. Ren X, Ren L, Wei Q, Shao H, Chen L, Liu N. Advanced glycation end-products decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells. Cardiovasc Diabetol. 2017;16(1):52. doi: https://doi.org/10.1186/s12933-017-0531-9
4. Saliba W, Barnett-Griness O, Gronich N, Molad J, Naftali J, Rennert G, Auriel E. Association of Diabetes and Glycated Hemoglobin With the Risk of Intracerebral Hemorrhage: A Population-Based Cohort Study. Diabetes Care. 2019;42(4):682-8. doi: https://doi.org/10.2337/dc18-2472
5. Chen J, Xu X-M, Xu Z, Zhang J. Animal Models of Acute Neurological Injuries. Springer International

Publishing. 2nd ed. Editors J Chen, X-M Xu, Z Xu. J Zhang. 2019. p. 544. doi: https://doi.org/10.1007/978-3-030-16082-1

6. Nafisa A, Gray SG, Cao Y, Wang T, Xu S, Wattoo FH, Barras M, Cohen N, Kamato D, Little PJ. Endothelial function and dysfunction: Impact of metformin. Pharmacol Ther. 2018;192:150-62.
doi: https://doi.org/10.1016/j.pharmthera.2018.07.007

7. Potârniche AV, Dreancă AI, Sarpataki O, Sevastre B, Marcus I. Experimental model of Streptozotocin-Nicotinamide induced Diabetes Mellitus type II in Sprague-Dawley rats: Step by step protocol and the encountered issues Rev Rom Med Vet. 2018;28(2):22-26.

8. Gabbay KH, Sosenko JM, Banuchi GA, Mininsohn MJ, Flückiger R. Glycosylated hemoglobins: increased glycosylation of hemoglobin A in diabetic patients. Diabetes. 1979;28(4):337-40.
doi: https://doi.org/10.2337/diab.28.4.337

9. Nair D, Nedungadi D, Mishra N, Nair BG, Nair SS. Identification of carbonylated proteins from monocytic cells under diabetes-induced stress conditions. Biomed Chromatogr. 2021;35(6):e5065.
doi: https://doi.org/10.1002/bmc.5065

10. Lancellotti P, Ancion A, D'Orio V, Gach O, Maréchal P, Krzesinski JM. [Bradykinin and cardiovascular protection. Role of perindopril, an inhibitor of angiotensin conversion enzyme]. Rev Med Liege. 2018;73(4):197-205. French.

11. An H, Wei R, Ke J, Yang J, Liu Y, Wang X, Wang G, Hong T. Metformin attenuates fluctuating glucose-induced endothelial dysfunction through enhancing GTPCH1-mediated eNOS recoupling and inhibiting NADPH oxidase. J Diabetes Complications. 2016;30(6):1017-24.
doi: https://doi.org/10.1016/j.jdiacomp.2016.04.018

12. Münch G, Keis R, Wessels A, Riederer P, Bahner U, Heidland A, Niwa T, Lemke HD, Schinzel R. Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. Eur J Clin Chem Clin Biochem. 1997;35(9):669-77.
doi: https://doi.org/10.1515/cclm.1997.35.9.669

13. Shi Y, Vanhoutte PM. Macro- and microvascular endothelial dysfunction in diabetes. J Diabetes. 2017;9(5):434-49.
doi: https://doi.org/10.1111/1753-0407.12521

14. Su JB. Vascular endothelial dysfunction and pharmacological treatment. World J Cardiol. 2015;7(11):719-41. doi: https://doi.org/10.4330/wjc.v7.i11.719

15. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. Oxid Med Cell Longev. 2020;2020:8609213.
doi: https://doi.org/10.1155/2020/8609213

СПИСОК ЛІТЕРАТУРИ

1. Антомонов М. Ю. Математическая обработка и анализ медико-биологических данных. Киев: Малий друк, 2006. 558 с

2. A Review of the Role of Bradykinin and Nitric Oxide in the Cardioprotective Action of Angiotensin-Converting Enzyme Inhibitors: Focus on Perindopril / A. Ancion et al. *Cardiol Ther.* 2019. Vol. 8, No. 2. P. 179-191.
DOI: https://doi.org/10.1007/s40119-019-00150-w

3. Advanced glycation end-products decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells / X. Ren et al. *Cardiovasc Diabetol*. 2017. Vol. 16, No 52. DOI: https://doi.org/10.1186/s12933-017-0531-9

4. Association of Diabetes and Glycated Hemoglobin With the Risk of Intracerebral Hemorrhage: A Population-Based Cohort Study / W. Saliba et al. *Diabetes Care.* 2019. Vol. 42, No. 4. P. 682-688. DOI: https://doi.org/10.2337/dc18-2472

5. Chen J., Xu Z. C., Xu X.-M., Zhang J. H. Animal Models of Acute Neurological Injury II [electronic resource]. 2nd ed. *Springer International Publishing*. 2019. 544 p.
DOI: https://doi.org/10.1007/978-3-030-16082-1

6. Endothelial function and dysfunction: Impact of metformin / A. Nafisa et al. *Pharmacol Ther.* 2018. Vol. 192. P. 150-162.
DOI: https://doi.org/10.1016/j.pharmthera.2018.07.007

7. Experimental model of Streptozotocin-Nicotinamide induced Diabetes Mellitus type II in Sprague-Dawley rats: Step by step protocol and the encountered issues / A. V. Potârniche et al. *Rev Rom Med Vet.* 2018. Vol. 28, No. 2. P. 22-26

8. Glycosylated hemoglobins: increased glycosylation of hemoglobin A in diabetic patients / K. H. Gabbay et al. *Diabetes*. 1979. Vol. 28, No. 4. P. 337-340. DOI: https://doi.org/10.2337/diab.28.4.337

9. Identification of carbonylated proteins from monocytic cells under diabetes-induced stress conditions / D. Nair et al. *Biomed Chromatogr*. 2021. Vol. 35, No. 6. e5065. DOI: https://doi.org/10.1002/bmc.5065

10. Lancellotti P. Bradykinine et protection cardiovasculaire. Rôle du périndopril, un inhibiteur de l'enzyme de conversion de l'angiotensine. *Rev Med Liege*. 2018. Vol. 73, No. 4. P. 197-205

11. Metformin attenuates fluctuating glucose-induced endothelial dysfunction through enhancing GTPCH1-mediated eNOS recoupling and inhibiting NADPH oxidase / H. An et al. *J Diabetes Complications*. 2016. Vol. 30, No. 6. P. 1017-1024.
DOI: https://doi.org/10.1016/j.jdiacomp.2016.04.018

12. Münch G. Determination of advanced glycation end-products in serum by fluorescence spectroscopy and competitive ELISA. *Eur J Clin Chem Clin Biochem.* 1997. Vol. 35, No. 9. P. 669-677.
DOI: https://doi.org/10.1515/cclm.1997.35.9.669

13. Shi Y., Vanhoutte P. M. Macro- and microvascular endothelial dysfunction in diabetes. *J Diabetes*. 2017. Vol. 9, No. 5. P. 434-449.
DOI: https://doi.org/10.1111/1753-0407.12521

14. Su J. B. Vascular endothelial dysfunction and pharmacological treatment. *World J Cardiol*. 2015. Vol. 7, No. 11. P. 719-741.
DOI: https://doi.org/10.4330/wjc.v7.i11.719

15. Yaribeygi H., Sathyapalan T., Atkin S. L., Sahebkar A. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxid Med Cell Longev*. 2020. 8609213.
DOI: https://doi.org/10.1155/2020/8609213

The article was received
2021.07.08