LIPID METABOLIC CHANGES IN RAT BRAIN DURING PERMANENT CEREBRAL ISCHEMIA

Kostyuk AI[1], Kotova DA[1,2], Demidovich AD[1,3], Panova AS[1], Kelmanson IV[1,4], Belousov VV[1,4] ✉, Bilan DS[1] ✉

[1] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

[2] Lomonosov Moscow State University, Moscow, Russia

[3] Lomonosov Moscow State University, Moscow, Russia

[4] The Research Institute for Translational Medicine,
  Pirogov Russian National Research Medical University, Moscow, Russia

With each year, millions of people remain targeted by brain stroke, it still is by all means a global concern of the mankind. Despite all efforts to understand this disease better, there is still a lack of information on pathophysiology of ischemic stroke. Scrutinized data on biochemical changes at early stages of ischemia may help understand the mechanisms of the disorder and possibly reveal ways to finding the cure. The key role in the pathogenesis of stroke belongs to lipids as well as to the molecules associated with their biosynthesis and functionality. On the one hand, stroke evokes a deep oxidative stress leading to damage to biomolecules including lipids while on the other hand, due to the lack of reducing equivalents, the cellular biosynthesis processes are interrupted. The focus of this work was to study the changes taking place in the tissues of rat brain as a result of ischemia including estimation of levels of total cholesterol, FFA, MDA, GSH, and NADP(H). It was shown that in 24 hours from the onset of ischemia, there was a significant decrease in levels of FFA, total cholesterol and GSH, and an increase in the level of MDA, a marker of lipid peroxidation. NADP(H) pool level decreased twice in 6 hours from MCAO.

✉ Correspondence should be addressed: Vsevolod V. Belousov
Miklukho-Maklaya, 16/10, Mosow, 117997; vsevolod.belousov@gmail.com

Dmitry S. Bilan
Miklukho-Maklaya, 16/10, Mosow, 117997; d.s.bilan@gmail.com

ИЗМЕНЕНИЕ КЛЮЧЕВЫХ ПАРАМЕТРОВ МЕТАБОЛИЗМА ЛИПИДОВ В ТКАНЯХ МОЗГА КРЫС ПРИ ПЕРМАНЕНТНОЙ ИШЕМИИ

А. И. Костюк[1], Д. А. Котова[1,2], А. Д. Демидович[1,3], А. С. Панова[1], И. В. Кельмансон[1,4], В. В. Белоусов[1,4] ✉, Д. С. Билан[1] ✉

[1] Институт биоорганической химии имени М. М. Шемякина и Ю. А. Овчинникова, Москва, Россия

[2] Биологический факультет, Московский государственный университет имени М. В. Ломоносова, Москва, Россия

[3] Химический факультет, Московский государственный университет имени М. В. Ломоносова, Москва, Россия

[4] Научно-исследовательский институт трансляционной медицины,
  Российский национальный исследовательский медицинский университет имени Н. И. Пирогова, Москва, Россия

Инсульт головного мозга является важнейшей проблемой современного человечества, миллионы людей ежегодно становятся жертвами этого недуга. Несмотря на масштабные исследования в этой области, до сих пор существует недостаток информации о патофизиологии ишемического инсульта. Тщательное изучение изменений биохимических показателей на раннем этапе заболевания позволит установить механизм его протекания и в будущем найти эффективное решение проблемы. Ключевая роль в патогенезе инсульта принадлежит липидам и молекулам, ассоциированным с их биосинтезом и поддержанием их функционального состояния. С одной стороны, при патогенезе инсульта происходит мощный окислительный стресс, который приводит к повреждению молекул, в том числе липидного состава. С другой стороны, из-за нехватки восстановительных эквивалентов останавливается биосинтез новых молекул взамен поврежденных. Целью работы было исследовать изменения в тканях мозга крыс при ишемии таких параметров, как концентрации общего холестерина, свободных жирных кислот (СЖК), малонового диальдегида (МДА), а также уровней восстановленного глутатиона (GSH) и общего НАДФ(Н). В результате исследования выявлено, что через 24 ч с момента начала развития ишемии уровни СЖК, холестерина и GSH существенно снижаются, при этом уровень МДА, маркера перекисного повреждения липидов, увеличивается. Уровень общего пула НАДФ(Н) снижается в 2 раза уже через 6 ч с момента окклюзии сосуда.

✉ Для корреспонденции: Всеволод Вадимович Белоусов
ул. Миклухо-Маклая, д. 16/10, г. Москва, 117997; vsevolod.belousov@gmail.com

Дмитрий Сергеевич Билан
ул. Миклухо-Маклая, д. 16/10, г. Москва, 117997; d.s.bilan@gmail.com

According to the World Health Organization (WHO), stroke is the second leading cause of death worldwide. Each year more than 15 million people worldwide suffer stroke. Of these, 6 million die and another 5 million are permanently disabled [1]. The ischemic stroke is caused by angiostenosis or an abrupt blockage of cerebral arteries with resulting reduction incerebral perfusion and rapid damage of brain tissues [2].

Recent studies show biochemical changes occurring in brain cells during ischemia. In particular, hypoxia increases extracellular concentration of glutamate, an important neurotransmitter, and its binding to glutamate receptors [3] leading to a sharp increase of intracellular calcium level. Uncontrolled Ca^{2+} regulation results in activation of Ca^{2+}-dependent proteins and upsetting the regulation of cellular cascades [4]. Biochemical changes result in reductions in cell pH and ATP production as well as changes in intracellular ionic ratios [5, 6]. At later stages of pathogenesis, DNA, proteins and lipids are damaged [7]. The pathologic events culminate in mitochondrial dysfunction and extensive cell death [8].

Lipids constitute about half of brain tissue dry weight and have important structural and metabolic functions. Glycerophospholipids and sphingolipids are major components of cellular membranes; cholesterol is a key component of lipid rafts, dynamic assemblies in the cell membrane, regulating membrane motility and signal transduction. Since lipids cannot pass through a blood-brain barrier (BBB), de novo synthesis of lipids occurs in nervous cells. NADPH plays the essential role in the lipid biosynthesis acting a reducing equivalent in anabolic reactions.

Ischemia-reperfusion induces overproduction of reactive oxygen species (ROS) [9] leading to lipid peroxidation. Consequently, there are increased levels of lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal [10] that can be used as markers of cellular oxidative stress.

In response to intracellular ROS increase, antioxidative defense mechanisms are activated in cells. Glutathione (GSH) plays a key role in thiol-disulfide exchange reactions [11, 12] while maintaining the redox state of the glutathione pool is mediated by NADPH-dependent systems [12]. Thus, both parameters are important in the regulation of protection processes against cell damage caused by oxidative stress during ischemia [13].

In our study, we estimated levels of the most important biochemical compounds related to lipid metabolism in rat brain tissues during ischemic damage. Measurements of metabolite concentrations were performed using commercially available kits and standard protocols (MDA, NADP(H)) [14, 15].

METHODS

The study was carried out on male Wistar rats (weight ranging from 280 g to 330 g) obtained from Pushchino breeding facility (Moscow region; Russia). Animals were housed under standard vivarium conditions in plastic cages, three per cage. The animals had free access to food and water. The experimental work (surgeries and biochemical tests) was carried out within a month (June, 2018).

A middle cerebral artery occlusion (MCAO) was performed as described in [16]. Inhalation anesthesia with a mixture of isoflurane with air was used (Aerrane by Baxter; USA): 5% concentration for induction and 1.5% concentration for maintenance of general anesthesia.

The animals were analgesized with 5 mg/kg ketoprofen (Ketonal by Sandoz; Switzerland) administered subcutaneously; local analgesia was induced by administering 2% Novocaine.

0.185 mm MCAO (middle cerebral artery occlusion) sutures were used (403756PK10Re, Doccol; USA).

Tissue sample preparation

The animals were randomized into five groups, four individuals each. The control group included intact animals. In the other four groups, the animals were subjected to permanent right middle cerebral artery occlusion (MCAO). Biochemical analysis was carried out at 5 time points: 1 hour (Group 2), 3 hours (Group 3), 6 hours (Group 4), and 24 hours (Group 5) post-ischemia.

Following 1 to 24 hours of occlusion, the animals were sacrificed and the brains were removed. Brain tissue without cerebellum and olfactory bulbs was frozen immediately in liquid nitrogen and then homogenized thoroughly. The received material was weighed and stored at –70 °C until further processing.

Measurement of FFA level

The quantification of free fatty acids in brain samples was performed with a Free Fatty Acid Quantification Assay Kit (Abcam; Cambridge, UK). At first, the free fatty are being converted to acyl-CoA under acyl-CoA synthetase action. Then specific enzyme oxidizes the obtained monothioesters that is followed by reduction of colourless dye with formation of fluorescent compound (Ex. 535 nanometers; Em. 587 nanometers).

Tissue samples were thawed on ice and then homogenized in 200 μL 1% Triton X-100 in pure chloroform using a manual Potter homogenizer. The obtained homogenate was incubated on ice for 30 minutes to increase the efficiency of extraction. The extracts were centrifuged for 10 minutes at maximum speed, and after that, the organic phase was collected. To remove chloroform, the samples were air dried at 50 °C in a fume hood for an hour and then placed in vacuum dryer for the same time. The obtained lipid fraction was dissolved in 200 μL Fatty Acid Assay Buffer by extensive vortexing.

Aliquots with volumes from 10 to 40 uL were used for the assay. Sample volumes were adjusted to 50 μL with Fatty Acid Assay Buffer solution. Also, a set of palmitic acid standards was prepared for standard curve plotting. 2 μL ACS Reagent (the one with acyl-CoA synthetase activity) was added to each standard and sample well with subsequent incubation at 37 °C for 30 minutes. After that, 50 μL of Reaction mix (45.6 μL Assay Buffer; 0.4 μL Fatty Acid Probe; 2 μL Enzyme Mix; 2 μL Enchancer) were added to each well, with following incubation at 37 °C for 30 minutes.

Fluorescence was measured using Tecan Infinite 200 Pro plate reader (Tecan; Switzerland) at Ex/Em = 535/587 nm. Based on standard readings, the dependence of fluorescence intensity on FFA content in the samples was determined. Based on that, concentrations of FFA in the test samples were calculated. The obtained values were normalized to corresponding tissue weight.

Measurement of cholesterol level

Total cholesterol level (both free cholesterol and cholesteryl esters) in brain samples was quantified using commercially available Cholesterol/Cholesteryl Ester Detection Kit (Abcam; UK) according to the related protocol. In the assay, free cholesterol is oxidized by cholesterol dehydrogenase to generate NADH which reacts with a sensitive probe resulting in strong absorbance at 450 nm.

Tissue samples were thawed on ice and then homogenized in 200 µL CHCl3 : IPA : 10% NP-40 (7 : 11 : 0.1) using a manual Potter homogenizer. Each sample was centrifuged for 10 minutes at maximum speed, and after that, the organic phase was collected. To remove chloroform, the samples were air dried at 50 °C in a fume hood for an hour and then placed in vacuum dryer for the same time. The received lipid fraction was dissolved in 200 µL Cholesterol Assay Buffer by extensive vortexing.

Aliquots with volumes from 10 to 40 uL were used for the assay. Sample volumes were adjusted to 50 µL with Cholesterol Assay Buffer. A set of cholesterol standards was prepared for standard curve plotting. 2 µL Esterase (enzyme that hydrolyzes cholesterol esters to cholesterol) was added to each standard and sample well with following incubation at 37 °C for 30 minutes. After that, 48 µL of Reaction mix (44 µL Cholesterol Assay Buffer, 2 µL Substrate Mix, 2 µL Cholesterol Enzyme Mix) was added to each well, with subsequent incubation at 37 °C for 30 minutes.

The absorbance was measured using Tecan Infinite 200 Pro (Tecan; Switzerland) at 450 nm. Based on standard readings, the dependence of fluorescence intensity on cholesterol content in the samples was determined. After that the concentrations of total cholesterol in the test samples were calculated. The obtained values were normalized to corresponding tissue weight.

Measurement of NADP(H) level

NADP(H) content in brain samples was determined using nitro blue tetrazolium (NBT) and phenazine methosulfate (PMS). Acting as a donor of electrons, NADPH reduces NBT in the presence of PMS converting it into formazan that absorbs at 620 nm. To visualize presence of $NADP^+$, an enzymatic reaction was used where glucose-6-phosphate dehydrogenase (G6PDHs) oxidized glucose-6-phosphate with reduction of cofactor.

About 300 mg of brain tissue were thawed on ice and homogenized using a manual Potter homogenizer in the presence of 1.5 ml of previously cooled carbonate buffer (100 мМ Na_2CO_3, 20 мМ $NaHCO_3$, 20 мМ nicotinamide). The samples were centrifuged at 4 °C for 5 min at maximum speed. 10 µl were taken out from the supernatant to determine the protein concentration in the solution while the remaining volume was further refined using Amicon® Ultra 0,5 mL 10 K columns (Merck Millipore; Germany) in accordance with the producer's protocol.

For analysis, the samples were placed to a 96-well plate in an amount of 10 µl/well. A standard series of solutions with known NADP(H) content was prepared. Samples were brought up to 100 µl with a reaction mixture of the following composition (per 10 reactions): 540 µl MQ, 100 µl 1M TRIS-HCl buffer (pH 8.0), 50 µl of 10 mM NBT, 150 µl of 10 mM PMS, 50 µl of a 100 mM EDTA solution, 10 µl of 100 mM glucose-6-phosphate. Samples were incubated at +37 ° C for 5 min, following which the reaction was initiated by adding 1 µl of 250 U/ml glucose-6-phosphate dehydrogenase.

Absorbance at 620 nm was measured for each sample using Tecan Infinite 200 Pro plate reader (Tecan; Switzerland) in 10 min sessions. Each probe analysis was repeated without enzyme for the determination of background formazan formation under the action of cell lysate components. The dependence of the reaction rate on the content of NADP(H) in a sample was determined. The resulting calibration graph was used to determine the concentration of total NADP(H) in the experimental probes. The values obtained were normalized to the protein concentration.

Measurement of malondialdehyde level

To determine the intensity of lipid peroxidation in the samples, a modified TBARS assay was used. Malondialdehyde, which is one of the key products of lipid peroxidation, reacts with two 2-thiobarbituric acid (TBA) molecules to form a colored compound that intensively absorbs at 532 nm.

Approximately 100 mg of tissue were thawed on ice and homogenized in 750 µl of previously cooled MQ-grade pure water using a manual Potter homogenizer. 5 µl of butylated hydroxytoluene dissolved in methanol at a 10% concentration (w/v) were added to water to prevent *ex vivo* oxidation. The samples were centrifuged at 3000 × g for 15 min at 4 °C to obtain a soluble fraction containing cell membranes. At this stage, 10 µl were removed from supernatant for further assessing protein concentration. 250 µl of 40% trichloroacetic acid (TCA) were added to 350 µl of samples for precipitation of proteins and stirred vigorously. The samples were again centrifuged at 3000 × g for 15 min at 4 °C.

For analysis, 500 µl supernatant volume was mixed with 500 µl of 0.67% TBA in 0.05 N NaOH. To prepare a standard solution series, 0.5 mM 1,1,3,3,-tetramethoxypropane (TMP) dissolved successively in 1 ml of 96% ethanol and 49 ml of MQ-grade water was used. Standard solutions were mixed with 500 µl of 0.67% TBA in 0.05 N NaOH, after which all samples were incubated at +100 °C for 15 minutes. After the incubation, aliquots of 200 µl were placed in a 96-well plate, and absorbance at 532 nm was measured using Tecan Infinite 200 Pro (Tecan; Switzerland). A calibration graph was created in accordance with the standard series samples, and MDA content was determined accordingly. The values obtained were normalized to the protein concentration.

Measurement of reduced glutathione (GSH) level

GSH in brain samples was quantified using commercially GSH/GSSG Ratio Detection Assay Kit, (Fluorometric-Green Abcam; UK). In the assay, non-fluorescent Thiol Green dye becomes strongly fluorescent upon reacting with GSH (Ex. 490, Em. 520).

Tissue material was thawed on ice and homogenized in 400 µl of phosphate-saline buffer (pH 7.4) containing 0.5% NP-40 using a manual Potter homogenizer. The homogenates were centrifuged at maximum speed for 15 min at +4 °C. At the next stage, the samples were deproteinized by adding 1/5 volume of 100% (w/v) trichloroacetic acid (TCA). After intensive mixing and incubation on ice for 10 min, the samples were again centrifuged at 12,000 × g for 5 min at +4 °C. The TCA was neutralized by adding a $NaHCO_3$ solution to the samples. Finally, the last centrifugation was carried out at 13,000 × g and +4 °C for 15 minutes.

For analysis, the supernatants were diluted by 10 times with Assay Buffer solution, and aliquots of 50 µl of each sample were placed in a 96-well plate. A set of standards with known concentrations of GSH was also prepared. 50 µl of Thiol Green solution were diluted by 100 times with Assay Buffer and then added to samples. The samples were incubated in the dark for one hour.

Fluorescence was measured using Tecan Infinite® 200 PRO (Tecan; Switzerland). Based on standard curve data, dependence of fluorescence intensity on the amount of glutathione in the sample was determined. The calibration graph was used to determine GSH content in each sample. The values obtained were normalized to starting tissue weight.

Measurement of protein concentration

To determine concentration of proteins, a commercially available Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich; Germany) was used. In each series of experiment, a set of standards with known concentration of bovine serum albumin (BSA) protein was prepared. A mixture containing 1 part of 4% (w/v) copper (II) sulfate pentahydrate and 50 parts of the bicinchononic acid solution was added to each sample. The samples were incubated at +37 °C for half an hour.

Absorbance at 562 nm was measured using Tecan Infinite 200 Pro (Tecan; Switzerland). Using standard curve, protein concentrations were estimated for each sample.

RESULTS

A significant change in NADP(H) pool level was observed in 6 hours after MCAO. For control group, it was 10.64 ± 0.57 µmol/mg of protein while for damaged brain area, it was 4.45 ± 0.79 µmol/mg of protein. In 24 hours after MCAO, NADP(H) level was 1.57 ± 0.46 µmol/mg of protein that is about 7 times lower as compared to control group (Fig. 1A).

Since NADP(H) is involved in many biosynthetic processes including the lipid metabolism, we expected to observe a significant decrease in cholesterol and fatty acids levels in brain tissues. In our experiment, the level of total cholesterol (both free cholesterol and cholesteryl esters) began to decrease in 6 h after MCAO: 6.85 ± 0.1 µg/mg of tissue while control value was 7.29 ± 0.20 µg/mg of tissue accordingly. In 24 hours after MCAO, the total cholesterol level in damaged tissues continued to decrease to value of 5.95 ± 0.4 µg/mg of tissue (Fig. 1B). For Group 3 (3 hours after MCAO), no difference in cholesterol level was observed as compared to control group. However, since a significant variation of values was observed for animals in Group 3, it is likely that 3 hours after MCAO, the cholesterol level started to decrease in damaged tissues.

The level of the free fatty acids (FFA) in damaged brain tissues differed from related control level in Group 5 (24 hours from MCAO) only: 0.25 ± 0.003 nmol/mg vs 0.31 ± 0.01 nmol/mg (Fig. 1C).

Malondialdehyde (MDA) is one of the lipid peroxidation markers. The tissue injury causes a slowdown in cell biosynthetic processes resulting in accumulation of biodegradation products accordingly. In control group, the MDA level was 846.23 ± 63.41 pmol/mg protein showing a normal functional level of cell biodegradation processes. A pathology occurs when cells fail to withstand the damage and maintain proper biosynthesis levels. In 24 hours after MCAO, MDA level in damaged brain tissues increased to 1272.61 ± 124.47 pmol/mg protein (Fig. 1D) in ischemic tissue. At this time an extensive cell death occurred that can be visualized by staining of brain slices using specific dye such as 2,3,5-triphenyltetrazolium chloride (TTC).
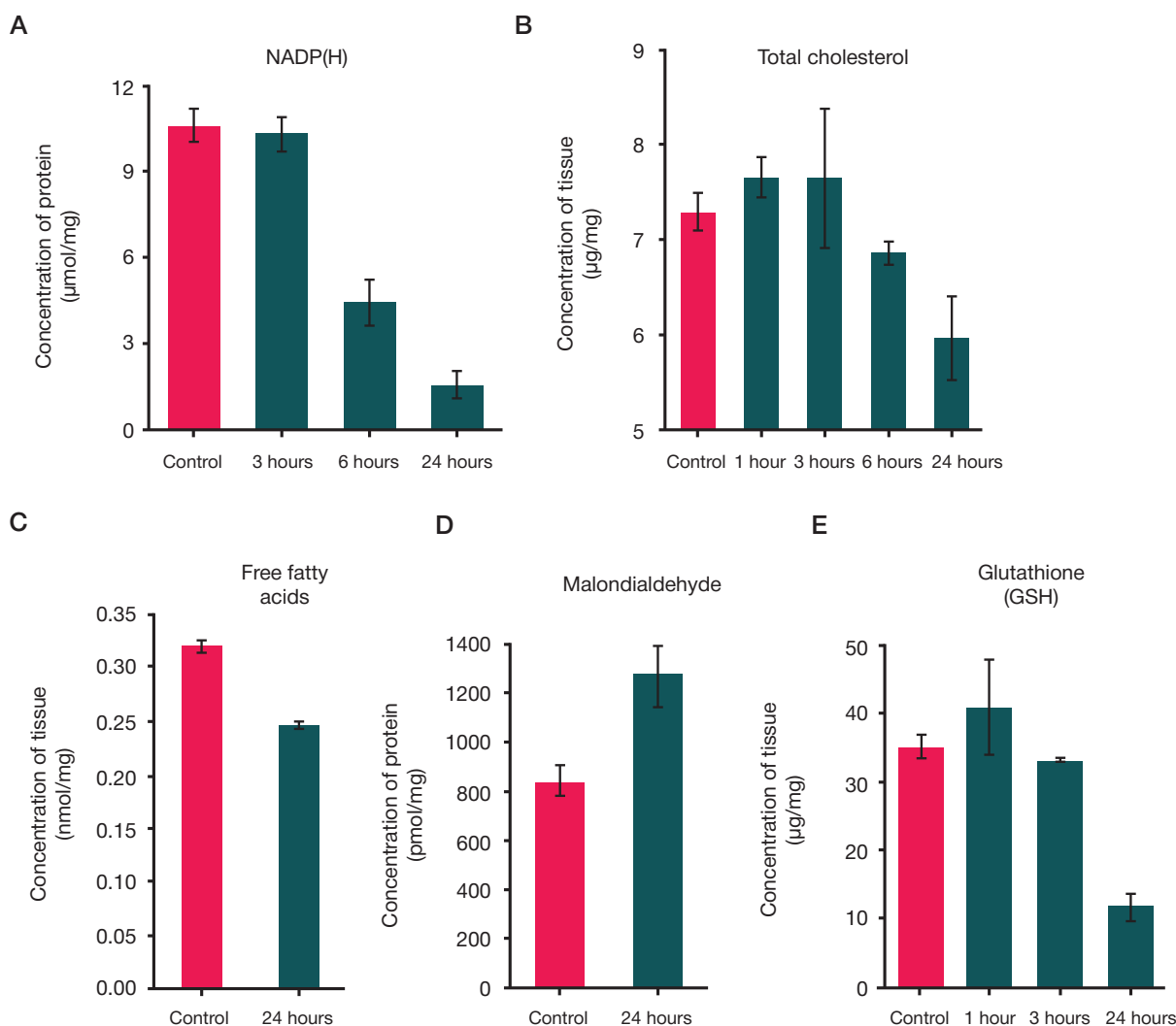

Fig. 1. Changes of biochemical parameters of brain tissues during permanent ischemia in rats. (**A**) NAD(H), (**B**) Total cholesterol, (**C**) Free fatty acids (FFA), (**D**) Malondialdehyde (MDA), (**E**) Glutathione (GSH)

The glutathione pool is an important redox component in cells. In particular, GSH is used by antioxidant enzymes such as glutathione peroxidase to neutralize ROS. Maintaining optimum GSH/GSSG ratio is essential to cell viability. NADPH is required for the regeneration of the reduced form of glutathione (GSH). In our study, no change was observed in the GSH levels for Groups 2–4 (within 6 hours from MCAO). In 24 hours after MCAO, GSH level in damaged brain tissues decreased to 11.8 µg/mg compared to 35.1 µg/ mg in the control group (Fig. 1E).

Thus, levels of FFA, total cholesterol, GSH and MDA changed significantly in 24 hours after MCAO while the level of NADP(H) was found to decrease in 6 hours after MCAO. X-axis: Time, hours (after MCAO). Error levels correspond to a standard error of an average. 4 animals per group.

DISCUSSION

Cerebral ischemic injury is a stress accompanied by structural damage at molecular or cellular level. Protection of tissues from the effects of ischemic damage occurs in different ways at many levels — through prevention of damage, repair and replacement of damaged structures.

Ischemic stroke is known to be accompanied by excessive and unregulated production of ROS [9] that are highly reactive compounds interacting with various cell compounds. One key target of ROS is lipids — both free and located within cell membranes. Lipid peroxidation is one of the common ways for the interaction of lipids and ROS. The process is a radical chain reaction, the end products of which are covalent aggregates of lipids as well as lipid hydroperoxides and lipid endoperoxides formed in the presence of oxygen [10]. The latter join intramolecular rearrangements with subsequent fragmentation of hydrocarbon tails. It is therefore feasible that an oxidative stress, while in the lipid phase, leads to an increase in the concentration of low molecular weight carbonyl compounds such as malondialdehyde (MDA). Most of the methods aimed at identifying oxidative stress are at least in some way based on registration of compounds of the above mentioned class [17, 18].

Interaction of lipids with reactive oxygen species may lead to a release of free fatty acids. Cell death in the foci of ischemia can also be followed by lipid hydrolysis [19]. It is known that free fatty acids can undergo auto-oxidation. Moreover, the cyclooxygenase pathway in the metabolism of arachidonic acid involves lipid hydroperoxides leading directly to the formation of oxygen radicals [10, 20]. Summing up the above, each and all of the mentioned factors will lead to an even greater contribution to peroxidation.

In practice, in many studies involving patients or laboratory animals, detection of MDA level in blood is performed. It was shown that MDA level increased significantly during the first 24 hours of ischemia, and it remained elevated for up to one week. A correlation of MDA level with a mortality rate has been shown for stroke patients [21]. Assessment of the concentration of MDA in ischemic brain can provide valuable information on the extent of tissue damage [22].

In our study, a model of permanent occlusion of the middle cerebral artery in rats was used. Under given conditions it was possible to register a noticeable increase in the concentration of MDA in 24 hours after MCAO. To better investigate the process, further studies are required involving ischemia/ reperfusion model. Reperfusion is believed to be the key source of ROS generation. Cell death during permanent ischemia may be supposed to take place due to reasons other than lipid peroxidation; presence of dead tissue becomes visible

within a few hours after MCAO. In fact, the approach based on TBARS analysis may be subject to artifacts likely involved at the sample preparation stage. Addition of antioxidants like butilated hydroxytoluene reduces the formation of MDA in the presence of atmospheric oxygen, still does not completely eliminates it. Thus, detecting minor shifts of MDA concentration with reference to control probes may be very difficult.

The concentration of reduced glutathione (GSH) was used as one of the markers of the cell redox state. Similar to MDA, we did not detect any changes in GSH level during the active phase of ischemic damage. However, its concentration decreased significantly in 24 hours after MCAO. Since GSH is involved in thiol-disulfide exchange reactions, an increase in ROS level results in an increase of glutathione oxidized form due to activation of antioxidant enzymes. This suggests that a noticeable increase in occurrence of oxidized lipids with subsequent cell death may lead to a significant decrease of GSH level. Still, to confirm this, further investigations are necessary — specifically, using the ischemia/reperfusion model. On the one hand, prolonged peroxidation may lead to a depletion of the GSH pool; on the other hand, the shortage of GSH can drive the system to a state in which peroxidation (which until then could have been restrained) starts causing a damaging effect. Finally, decrease of GSH level may be not due to the functioning of antioxidant systems in membranes. For instance, tissue necrotization is characterized by inability to maintain the GSH pool due to poor functioning of related enzyme systems and the lack of reducing equivalents.

In our study, concentration of NADP(H) decreased by 2 times in the first 6 hours after MCAO and by almost 7 times in 24 hours after MCAO. The NADP(H) decrease is believed to have an adverse impact on the antioxidant systems performance as well as on the anabolic processes. As mentioned earlier, this factor is likely to contribute to the decrease of GSH pool as well as the increase of lipid peroxidation level.

Not all the lipids damaged during peroxidation can be repaired. We believe that during ischemic cerebral damage, anabolic pathways involved in lipid biosynthesis should be activated in order to replace damaged components. The NADP(H) abundance is known to be connected with an efficiency of the biosynthesis of fatty acids and cholesterol as these metabolic pathways require reducing equivalents. As it was mentioned, NADP(H) level changed during the experiment.

In a large number of lab and clinical studies, FFA level in blood plasma was detected as this index can be considered a potential biomarker for detection of early stages of ischemic stroke. Hydrolysis of brain phospholipids with release of FFA into blood stream occurs during a stroke that may be a reason of observing increased FFA level in blood in the first few hours [23]. Based on other research data available, there is a correlation between FFA occurrence and the severity of disorder: the higher the level of FFA, the higher severity level observed [24, 25]. Though changes in FFA profile are of substantial metabolic importance, there is still a lack of data related to brain tissues.

In this work, we detected FFA levels in brain samples at different time points. A significant decrease in FFA level was observed in 24 hours after MCAO; during the acute phase of stroke there was no difference between FFA levels in test groups and the control group. Thus, in our experiment no evidence was received in support of activation of the synthesis of fatty acids for replacement of damaged membrane components. A decrease in FFA level after 24 hours from MCAO is most likely due to cell death leading to depletion of NADP(H) content, disruption of the enzyme system and perhaps some other

factors. For the studied model of permanent ischemia in rats, it is likely that no accumulation of lipids hydrolyzed after cell death occurs in brain tissues.

Similar data were obtained with regard to cholesterol concentration however, in case of cholesterol the decrease was observed earlier — in 6 hours after MCAO. In comparison to the original level of metabolites, concentration of cholesterol and FFA decreased similarly in a time lapse of 24 hours after the occlusion (1.24 times for FFA and 1.27 times for cholesterol).

CONCLUSIONS

In the permanent cerebral ischemia experiment, the levels of total cholesterol, FFA, MDA, GSH, and NADP(H) in brain tissues of rats have been measured. It was shown that in 24 hours from the onset of ischemia, there was a significant decrease in levels of FFA, total cholesterol and GSH, and an increase in the level of MDA, a marker of lipid peroxidation. NADP(H) level decreased twice in 6 hours from MCAO.

References

1. World Health Organization. Top ten causes of death. Available from: http://www.who.int/mediacentre/factsheets/fs310/en/index.html (accessed August 19, 2012).
2. Hinkle JL, Guanci MM. Acute Ischemic Stroke Review. Journal of Neuroscience Nursing. 2007; 39 (5): 285–93. PubMed PMID: 17966295.
3. Krzyżanowska W, Pomierny B, Filip M, Pera J. Glutamate transporters in brain ischemia: to modulate or not? Acta Pharmacologica Sinica. 2014; 35 (4): 444–62. PubMed PMID: 24681894.
4. Berliocchi L, Bano D, Nicotera P. Ca^{2+} signals and death programmes in neurons. Philos Trans R Soc Lond B Biol Sci. 2005; 360 (1464): 2255–8. PubMed PMID: 16321795.
5. Klogeris T, Baines CP, Krenz M, Korthuis RJ. Ischemia/Reperfusion. Compr Physiol. 2016; (7): 113–70 PubMed PMID: 28135002.
6. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol. 2012; (298): 229–317. PubMed PMID: 22878108.
7. Allen CL, Bayraktutan U. Oxidative Stress and Its Role in the Pathogenesis of Ischaemic Stroke. International Journal of Stroke. 2009; 4 (6); 461–70. PubMed PMID: 19930058.
8. Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL. Mitochondrial dysfunction and myocardial ischemia-reperfusion: implications for novel therapies. Annual review of pharmacology and toxicology. 2017; (57): 535–65. PubMed PMID: 27860548.
9. Vanden Hoek TL, Li C, Shao Z, Schumacker PT, Becker LB. Significant levels of oxidants are generated by isolated cardiomyocytes during ischemia prior to reperfusion. J Mol Cell Cardiol. 1997; (29): 2571–83. PubMed PMID: 9299379.
10. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017; 482 (3): 419–25. PubMed PMID: 28212725.
11. Gaucher C, Boudier A, Bonetti J, Clarot I, Leroy P, Parent M. Glutathione: Antioxidant Properties Dedicated to Nanotechnologies. Antioxidants (Basel). 2018; 7 (5): 62. PubMed PMID: 29702624.
12. Bilan DS, Shohina AG, Lukjanov SA, Belousov VV. Osnovnye redoks-pary kletki. Bioorganicheskaya himija. 2015; (41): 385–402. Russian.
13. Dringen R, Pawlowski PG, Hirrlinger J. Peroxide detoxification by brain cells. Journal of Neuroscience Research. 2004; 79 (1–2): 157–165. PubMed PMID: 15573410.
14. Garcia YJ, Rodrıguez-Malaver AJ, Peñãloza N. Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. Journal of Neuroscience Methods. 2005; (144): 127–35. PubMed PMID: 15848246.
15. Charis WT, Scott MD. Single Extraction Method for the Spectrophotometric Quantification of Oxidized and Reduced Pyridine Nucleotides in Erythrocytes. Analytical Biochemistry. 1994; (222): 417–26. PubMed PMID: 7864367.
16. Uluc K, Miranpuri A, Kujoth GC, Akture E, Baskaya MK. Focal cerebral ischemia model by endovascular suture occlusion of the middle cerebral artery in the rat. J Vis Exp. 2011 Feb 5; (48). PubMed PMID: 21339721.
17. Gutteridge JMC, Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. Trends in Biochemical Sciences. 1990; 15 (4): 129–35. PubMed PMID: 2187293.
18. Ceconi C, Cargnoni A, Pasini E, Condorelli E, Curello S, Ferrari R. Evaluation of phospholipid peroxidation as malondialdehyde during myocardial ischemia and reperfusion injury. Am J Physiol. 1991; 260 (4 Pt 2): H1057–61. PubMed PMID: 2012211.
19. Bazan NG. Effects of ischemia and electroconvulsive shock on free fatty acid pool in the brain. Biochimica et Biophysica Acta (BBA) — Lipids and Lipid Metabolism. 1970; 218 (1): 1–10. PubMed PMID: 5473492.
20. Braughler JM, Hall ED. Central nervous system trauma and stroke. I. Biochemical considerations for oxygen radical formation and lipid peroxidation. Free Radic Biol Med. 1989; (6): 289–301. Pubmed PMID: 266366.
21. Cojocaru IM, Cojocaru M, Sapira V, Ionescu A. Evaluation of oxidative stress in patients with acute ischemic stroke. Rom J Intern Med. 2013 Apr-Jun; 51 (2): 97–106. Pubmed PMID: 24294813.
22. Lorente L, Martin MM, Perez-Cejas A, Abreu-Gonzalez P, Ramos L, Argueso M et al. Association between total antioxidant capacity and mortality in ischemic stroke patients. Ann Intensive Care. 2016; 6 (1): 39. Pubmed PMID: 27107565.
23. Golovko SA, Golovko MI. Plasma Unesterified Fatty-Acid Profile Is Dramatically and Acutely Changed under Ischemic Stroke in the Mouse Model. Lipids. 2018; 52 (6): 641–645. PubMed PMID: 30206953.
24. Niu Z, Hu H, Tang F. High Free Fatty Acid Levels Are Associated with Stroke Recurrence and Poor Functional Outcome in Chinese Patients with Ischemic Stroke. J Nutr Health Aging. 2017; 21 (10): 1102–1106. PubMed PMID: 29188867.
25. Wang X, Feng A, Zhu C. Cerebrospinal fluid levels of free fatty acid associated with ischemic stroke recurrence and functional outcome. Neurol Sci. 2016; 37 (9): 1525–9. PubMed PMID: 27245354.

Литература

1. World Health Organization. Top ten causes of death. Available from: http://www.who.int/mediacentre/factsheets/fs310/en/index.html (accessed August 19, 2012).
2. Hinkle JL, Guanci MM. Acute Ischemic Stroke Review. Journal of Neuroscience Nursing. 2007; 39 (5): 285–93. PubMed PMID: 17966295.
3. Krzyżanowska W, Pomierny B, Filip M, Pera J. Glutamate transporters in brain ischemia: to modulate or not? Acta

Pharmacologica Sinica. 2014; 35 (4): 444–62. PubMed PMID: 24681894.

4. Berliocchi L, Bano D, Nicotera P. Ca²⁺ signals and death programmes in neurons. Philos Trans R Soc Lond B Biol Sci. 2005; 360 (1464): 2255–8. PubMed PMID: 16321795.

5. Klogeris T, Baines CP, Krenz M, Korthuis RJ. Ischemia/ Reperfusion. Compr Physiol. 2016; (7): 113–70 PubMed PMID: 28135002.

6. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol. 2012; (298): 229–317. PubMed PMID: 22878108.

7. Allen CL, Bayraktutan U. Oxidative Stress and Its Role in the Pathogenesis of Ischaemic Stroke. International Journal of Stroke. 2009; 4 (6): 461–70. PubMed PMID: 19930058.

8. Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL. Mitochondrial dysfunction and myocardial ischemia-reperfusion: implications for novel therapies. Annual review of pharmacology and toxicology. 2017; (57): 535–65. PubMed PMID: 27860548.

9. Vanden Hoek TL, Li C, Shao Z, Schumacker PT, Becker LB. Significant levels of oxidants are generated by isolated cardiomyocytes during ischemia prior to reperfusion. J Mol Cell Cardiol. 1997; (29): 2571–83. PubMed PMID: 9299379.

10. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017; 482 (3): 419–25. PubMed PMID: 28212725.

11. Gaucher C, Boudier A, Bonetti J, Clarot I, Leroy P, Parent M. Glutathione: Antioxidant Properties Dedicated to Nanotechnologies. Antioxidants (Basel). 2018; 7 (5): 62. PubMed PMID: 29702624.

12. Билан Д. С., Шохина А. Г., Лукьянов С. А., Белоусов В. В. Основные редокс-пары клетки. Биоорганическая химия. 2015; (41): 385–402.

13. Dringen R, Pawlowski PG, Hirrlinger J. Peroxide detoxification by brain cells. Journal of Neuroscience Research. 2004; 79 (1–2): 157–165. PubMed PMID: 15573410.

14. Garcia YJ, Rodrıguez-Malaver AJ, Penãloza N. Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. Journal of Neuroscience Methods. 2005; (144): 127–35. PubMed PMID: 15848246.

15. Charis WT, Scott MD. Single Extraction Method for the Spectrophotometric Quantification of Oxidized and Reduced Pyridine Nucleotides in Erythrocytes. Analytical Biochemistry. 1994; (222): 417–26. PubMed PMID: 7864367.

16. Uluc K, Miranpuri A, Kujoth GC, Akture E, Baskaya MK. Focal cerebral ischemia model by endovascular suture occlusion of the middle cerebral artery in the rat. J Vis Exp. 2011 Feb 5; (48). PubMed PMID: 21339721.

17. Gutteridge JMC, Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. Trends in Biochemical Sciences. 1990; 15 (4): 129–35. PubMed PMID: 2187293.

18. Ceconi C, Cargnoni A, Pasini E, Condorelli E, Curello S, Ferrari R. Evaluation of phospholipid peroxidation as malondialdehyde during myocardial ischemia and reperfusion injury. Am J Physiol. 1991; 260 (4 Pt 2): H1057–61. PubMed PMID: 2012211.

19. Bazan NG. Effects of ischemia and electroconvulsive shock on free fatty acid pool in the brain. Biochimica et Biophysica Acta (BBA) — Lipids and Lipid Metabolism. 1970; 218 (1): 1–10. PubMed PMID: 5473492.

20. Braughler JM, Hall ED. Central nervous system trauma and stroke. I. Biochemical considerations for oxygen radical formation and lipid peroxidation. Free Radic Biol Med. 1989; (6): 289–301. Pubmed PMID: 266366.

21. Cojocaru IM, Cojocaru M, Sapira V, Ionescu A. Evaluation of oxidative stress in patients with acute ischemic stroke. Rom J Intern Med. 2013 Apr-Jun; 51 (2): 97–106. Pubmed PMID: 24294813.

22. Lorente L, Martin MM, Perez-Cejas A, Abreu-Gonzalez P, Ramos L, Argueso M et al. Association between total antioxidant capacity and mortality in ischemic stroke patients. Ann Intensive Care. 2016; 6 (1): 39. Pubmed PMID: 27107565.

23. Golovko SA, Golovko MI. Plasma Unesterified Fatty-Acid Profile Is Dramatically and Acutely Changed under Ischemic Stroke in the Mouse Model. Lipids. 2018; 52 (6): 641–645. PubMed PMID: 30206953.

24. Niu Z, Hu H, Tang F. High Free Fatty Acid Levels Are Associated with Stroke Recurrence and Poor Functional Outcome in Chinese Patients with Ischemic Stroke. J Nutr Health Aging. 2017; 21 (10): 1102–1106. PubMed PMID: 29188867.

25. Wang X, Feng A, Zhu C. Cerebrospinal fluid levels of free fatty acid associated with ischemic stroke recurrence and functional outcome. Neurol Sci. 2016; 37 (9): 1525–9. PubMed PMID: 27245354.