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Journal of Stem Cell Research & Therapeutics

Research Article

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Correlation between morphological, biochemical and functional indicators in acute ischemia-reperfusion rat brain

Abstract

Correlation analysis allows evaluating the statistical relationship between two random variables or two-dimensional data.

Aim: The aim of the study is to analyse the correlations between biochemical indicators determined in the somatosensory cortex and hippocampus, morphological manifestations of neuroapoptosis, and parameters of CNS functioning in acute cerebral ischemia in rats.

Materials and methods: To model an acute cerebral ischemia, ligatures were placed on the internal carotid artery bilaterally rats, after 20 minutes the blood flow through the specified vessels was restored. Experimental rat group received MSCs obtained from the human umbilical cord Wharton jelly at a dose 10^6 cells/rat. McGrow stroke-index scale, the functional state of the central nervous system, flow metric, immune histochemical and biochemical indicators in the tissue of the somatosensory cortex and hippocampus were analysed. Statistical processing of the results was carried out using the Statistica 7.0 computer program (StatSoft Inc., USA). Spearman's rank correlation analysis was performed to determine correlational dependencies.

Results: Biochemical indicators (in particular, lactate and glucose content, MDA, SDH, SOD and NOS activity determined in the brain tissue of the hippocampal and somatosensory cortical areas), reliably correlated with functional indicators of the central nervous system in rats both with and without brain ischemia.. After acute brain ischemia at day 7 (which corresponds to the sub-acute period of ischemia) specific direct correlation between protein content in brain tissue and morphological indicators appear as well as between protein content and neurological deficit both in the somatosensory cortex and in the hippocampus. At day 14 (the recovery period) after brain ischemia high specific inverse correlation takes place between MDA activity in the somatosensory cortex and morphological indicators in the hippocampus.

Conclusion: 1. Multiplex correlations between biochemical and morphological indicators determined in the somatosensory cortex and hippocampus indicate their close functional interaction. 2. No new specific correlations emerged when human umbilical cord Wharton jelly MSCs were transfused for neuro-protection immediately after brain reperfusion beginning, but some correlational dependencies disappeared.

Keywords: correlation analysis, ischemia-reperfusion, rats, somatosensory cortex, hippocampus, mesenchymal stromal cells

Volume 10 Issue 1 - 2025

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Received: March 4, 2025 | **Published:** April 22, 2025

Introduction

Cerebral ischemia is considered the leading cause of neurons and neuroglial cells death in the cerebral cortex and subcortical structures. Moreover, the brain cells destruction can be of both necrotic and apoptotic origin. The last one dominates in conditions of acute cessation in cerebral blood flow followed by restoration of its perfusion, which is associated with the occurrence of secondary reperfusion injuries.

Different ways of therapy are used to combat them, in particular, mesenchymal stromal cells (MSCs) are used to prevent and inhibit apoptosis. MSCs from the human umbilical cord are considered quite effective.[1]

The purpose of this research is to canvass the correlation dependences between morphological, functional and biochemical indicators, investigated in the somatosensory cortex and hippocampus of rats that underwent experimental short-term (20-minute) ischemia of a brain followed by its reperfusion (IR) and immediate introduction of MSCs obtained from the human umbilical cord jelly (at a dose -10^6 cells/rat) at once after brain reperfusion starting.

Materials and methods

70 sexually mature 4-month-old male rats of the Wistar line (weighing 160-190 g), grown in the vivarium of the Vinnytsia National Pirogov Memorial Medical University, were used for the study. The rats were kept under standard conditions with free access to water and food. To make the acute subtotal cerebral ischemia modelling, 60 rats were subjected to a 20-minute interruption of the blood supply to the brain (by placing ligatures on the internal carotid artery bilaterally) with subsequent restoration of blood flow through the indicated vessels (the so-called IR model), the surgical intervention was performed under anesthesia propofol ("Propofol-novo", Novofarm-Biosintez LLC production, Ukraine, at a dose 60 mg/kg). 10 rats formed up the control group (this is a group of sham-operated animals that underwent a skin incision under propofol anesthesia, preparation of blood vessels for ligature application, but without their ligation).

40 rats (taken from the specified 60 operated on) immediately after restoration of the brain blood supply were injected intravenously into the femoral vein with 0.9% NaCl solution (at a dose 2 ml/kg), while 20 rats received MSCs from the human umbilical cord jelly at a dose 106 cells/rat (thus formed the group of rats with an IR model (40 rats) and the group of rats (20 animals) with an IR model and subsequent introduction of MSCs from Wharton's jelly (as neuroprotective therapy). Detailed information about the used MSCs is provided in our previous publication.[2] In the process of performing the work, we were guided by the methodological recommendations of the Ukrainian Ministry of Health and the of bioethics' requirements in accordance with the National "General Ethical Principles of Animal Experiments" approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and the Law of Ukraine "On the Protection of Animals from Cruelty » dated February 26, 2006.

At day 7 and day 14 (the days correspond to ischemia periods – to the subacute and recovery periods, respectively), the rats were decapitated (anesthesia - pentobarbital ("Penbital", Bioveta production, Czech Republic, at the dose 100 mg/kg), their brains were ablated and biochemical, histoimmunochemical and morphological studies of the ablated rat brains were performed. In particular, the total number of neuron nuclei (per 1 mm^2) was counted in the somatosensory cortex and CA1 hippocampal zone, as well, as the ratio of the intact neuron nuclei number to the pathologically changed neuron nuclei number.[3] DNA (deoxyribonucleic acid) fragmentation level in neuron nuclei of the mentioned areas was studied by flow cytometry.[4] Neurological deficit in rats was determined according to the stroke-index scale of C.P. McGrow[5] in the subacute (day 7) and recovery (day 14) periods after IR modelling (or its imitation in the control group) to the same extent as the "open field" test (to analyse the functional state of the central nervous system).[6] Biochemical investigation included measuring of succinate dehydrogenase (SDH), glucose, lactate, pyruvate and malondialdehyde (MDA) content as well as superoxide dismutase (SOD) activity, total activity of NO synthases (NOS) and NADPH oxidase activity in rat brain tissue.[7-10]

To process the research results statistically the Statistica 7.0 computer program (StatSoft Inc. production, USA) was pitched upon. The non-parametric statistical methods were used (in particular, Spearman's non-parametric correlation rank analysis). Last one was taken to find the correlation relationships between variables and how strong those junctions were. As it is known a positive correlation result implies that both variables increase in relation to each other, while a negative correlation result denotes that as one variable decreases, the other increases. Nonparametric correlation analysis (Spearman's rank) we profit in cases where no assumptions can be made about the probability distribution. As a rule Spearman's rank correlation is used with qualitative data, but can be used with quantitative data.

To calculate the Spearman rank correlation the formula is applied (it counts the correlation coefficient r_s):

$$r_s = 1 - \frac{6\sum D^2}{n(n^2-1)}$$

r_s = Spearmanrank correlation

D = the difference between the ranks of corresponding variables

n = number of observations

Correlation coefficient r_s values can range from -1 to 1. A correlation coefficient of -1 describes a perfect negative, or inverse, correlation, with values in one series rising as those in the other decline (and vice versa). A coefficient of +1 shows a perfect positive correlation, or a direct relationship. A correlation coefficient of 0 connotes there is no linear relationship. After correlation coefficient r_s counting the p-value is calculated to determine whether the correlation coefficient is statistically significant.

Results

Using the nonparametric correlation analysis we made the canvass of dependences between morphological, biochemical and functional indicators in the hippocampus and somatosensory cortex for three groups of rats (for the group of sham-operated rats, the group of rats with IR modeling and the group of rats with IR modeling and immediate subsequent transfusion of MSCs from human umbilical cord jelly).

Group 1 (the sham-operated rats). There is a clear direct correlation (r_s = 0,99, p < 0,001) between glucose content in the somatosensory cortex (at day 7 after simulated surgery) and intact neurons number in the hippocampus (both at day 7 and day 14) (see the table 1). At the same time (day 7), the glucose content in the hippocampus is directly correlated with the percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the somatosensory cortex (DNA content = 2s, day 7, r = 0,90, p<0,05) and is inversely correlated with the percentage ratio of cells being in the G2 + M phase to all cells of a cell cycle in the somatosensory cortex (DNA = 4s, day 7, r_s = -0,90, p<0,05) and percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the hippocampus (DNA content = 2s, day 7, r_s = -0,97, p<0,01) . In addition, the glucose content in the cortex of the somatosensory area (measured at day 7) is inversely correlated with integrated fluorescence density of Iba+microglia on the frontal sections of the CA1 hippocampal zone (day 7), r_s = –0,90, p<0,05. At day 14 correlation dependences between the hippocampus and somatosensory cortex are also observed, thus glucose content in the cortex positively correlates with integral fluorescence density of Iba+microglia on the frontal slices of CA1 hippocampal zone (day 7, r_s = 0,90, p<0,05) and negatively – with integral fluorescence density of NeuN+ neurons on the frontal sections of the CA1 hippocampal zone (day 14, r_s = -0,90, p<0,05).

MDA content in the somatosensory cortex (at day 7) like the glucose content demonstrates a clear direct correlation link with a number of intact nuclei in the hippocampus (at day 7 and day14, r_s = 0,90, p<0,05 for both the dates). Besides this it inversely correlates with integral fluorescence density of GFAP+astrocytes on the frontal sections of CA1 hippocampal zone (day 7, r_s = -0,90, p<0,05) and latent period of ambulation in the peripheral squares in the "open field" test (at day 14, r_s = -0,79, p<0,05). At day 14 MDA content in the somatosensory cortex directly correlates with duration of ambulation episodes in peripheral squares (day 7, the "open field" test, r_s = 0,93, p<0,05).

At the same time SOD activity in the somatosensory cortex (day 7) displays negative (inverse) correlation with the number of intact nuclei in the somatosensory cortex (at days 7 and 14, r_s = -0,90, p<0,05 in both the cases).

Lactate content in the somatosensory cortex and hippocampus correlates with indexes of the "open field" test: at day 7 lactate content in the somatosensory cortex indirectly correlates with latent periods of grooming (r = r_s = -0,81, p<0,05) and latent periods of ambulation in peripheral squares (day 14, r_s = -0,77, p<0,05); in the hippocampus this index also shows an inverse dependence with latent period duration of the ambulation (day 7, r_s = -0,86, p<0,05), duration

of ambulation episodes in the peripheral squares (day 7, r_s = -0,85, p<0,05), latent period duration of the grooming (day 14, r_s = -0,82, p<0,05). At day 14 lactate content in the hippocampus positively correlates with grooming latent period (day 7, r_s = 0,85, p<0,05), number and duration of rearing episodes (day 7, r_s = 0,81 for both of them, p<0,05), latent period duration of rearing episodes (day 7, r_s = 0,77, p<0,05) and negatively – with latent period duration of the ambulation through the central squares (day 7, r_s = -0,81, p<0,05) and duration of the ambulation episodes through the peripheral squares (day 14, r_s = -0,86, p<0,05).

SDH activity in the somatosensory cortex shows a distinct correlation link to the flow cytometry results, for example at day7 it directly correlates with percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the somatosensory cortex (DNA content = 2s), r_s = 0,90, p<0,05); besides this it inversely correlates with percentage ratio of cells in the DNA synthesis phase to all cells of a cell cycle in the hippocampus (DNA content > 2s and < 4s) (at day 7, r_s = -0,90, p<0,05) and with percentage ratio of the cells in the G2 + M phase to all cells of a cell cycle in the somatosensory cortex (DNA = 4s) (at day 7, r_s = -0,90, p<0,05). At day 14 SDH activity in the somatosensory cortex has an inverse link to the percentage ratio of the DNA synthesis phase to all cells of the cell cycle ((DNA content > 2s and < 4s) in the somatosensory cortex (day 7, r_s = -0,90, p<0,05).

Nitric oxide synthase activity also correlates with some flow cytometry indexes. Thus, NOS activity in the somatosensory cortex (at day 7) positively correlates with percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the somatosensory cortex (DNA content = 2s) (day 7, r_s = 0,97, p<0,01) as well as with percentage ratio of G0G1 phase cells to all cells of a cell cycle in the somatosensory cortex (DNA content = 2s) (day 7, r_s = 0,97, p<0,001). Inverse correlations were found between NOS activity in the somatosensory cortex (day 7) and percentage of cells being in the G0G1 phase to all cells of a cell cycle in the hippocampus (DNA content = 2s) (day 7, = -0,95, p<0,05) and between NOS activity in the somatosensory cortex (day 7) and percentage ratio of the G2 + M phase cells to all cells of the cell cycle in the somatosensory cortex (DNA = 4s) (day 7, r_s = -0,97, p<0,01). Negative correlations also were found between NOS activity in the hippocampus (day 7) and percentage of G0G1 phase cells to all cells of a cell cycle in the hippocampus (DNA content = 2s) (day 7, r_s = -0,95, p<0,05) and between NOS activity in the hippocampus (day 7) and percentage ratio of the G2 + M phase cells to all cells of a cell cycle in the somatosensory cortex (DNA = 4s) (day 7, r_s = -0,97, p<0,01).

In 14 days after simulated surgery in the group of sham-operated rats we found the direct correlation links between NADPH oxidase activity in the somatosensory cortex (day 14) and percentage ratio of the G2 + M phase cells so all cells of the cell cycle in the somatosensory cortex (DNA = 4c) (day 7, r_s = 0,90, p<0,05) and between NADPH oxidase activity in the somatosensory cortex (day 14) and integral fluorescence density of GFAP+astrocytes on the frontal slices of CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05). NADPH oxidase activity in the somatosensory cortex (at day 14) negatively correlates with percentage ratio of cells in the G0G1 phase to all cells of the cell cycle in the somatosensory cortex (DNA content = 2s) (day 7, r_s = -0,90, p<0,05).

Protein content in the somatosensory cortex and hippocampus at day 14 shows a positive correlation with fluorescence density of RECA-1 positive blood vessels in the CA1 hippocampal zone (day 7, r = 0,90, p<0,05 for both the correlation links).

It is worth noting that we emphasized the most potent correlational dependencies in group 1.

Group 2 (the group of rats with IR modeling without any anti-ischemic therapy). In comparison with the sham-operated animals (which demonstrate a clear direct correlation (r_s = 0,99, p<0,001) between glucose content in the somatosensory cortex (at day 7) and intact neurons number in the hippocampus (both at day 7 and day 14)) the rats after transitory ischemia-reperfusion of a brain reveal a direct correlation dependence between glucose content in the somatosensory cortex (at day 7 after IR modelling) and number of damaged nuclei (karyopyknosis, karyorrhexis) in the hippocampus (day 14, r_s = 0,89, p<0,05) (see the tabl.2). At the same time (day 7) glucose content in the hippocampus is directly correlated with the percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the somatosensory cortex (DNA content = 2s, day 7, r_s = 0,90, p<0,05 (similarly to the rats of the group 1)) and with the intact nuclei number in the hippocampus (at day 14, r = 0,91, p<0,05). On the contrary the glucose content in the somatosensory cortex (measured at day 7) is inversely correlated with integral fluorescence density of RECA-1 positive blood vessels in CA1 hippocampal zone (day 7, r_s = -0,90, p<0,05), when in the group 1 it inversely correlates with integrated fluorescence density of Iba+microglia on the frontal sections of the CA1 hippocampal zone (day 7), r_s = -0,90, p<0,05.

In 14 days after IR modelling glucose content in the somatosensory cortex shows a negative link with integral fluorescence density of NeuN+ neurons in the frontal slices of CA1 hippocampal zone (day 7, r_s = -0,90, p<0,05), with integrated fluorescence density of GFAP+astrocytes on the frontal sections of the CA1 hippocampal zone (day 14, r_s = -0,90, p<0,05), with SUB-G0G1 regions on DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content < 2s in the somatosensory cortex (r_s = -0,90, p<0,05), where as glucose content in the hippocampus (at day 14) inversely correlates with percentage ratio of the G2 + M phase cells to all cells of a cell cycle in the somatosensory cortex (DNA = 4s) (day 7, r_s = -0,90, p<0,05), with the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex (day 7, r_s = -0,97, p<0,01) and with SUB-G0G1 areas on the DNA histograms - RN1 before the G0G1 peak (which indicates the cell nuclei with DNA content < 2s in the somatosensory cortex), r_s = -0,90, p<0,05.

After IR modeling in rats no correlation between MDA content in the somatosensory cortex (day 7) and a number of intact nuclei in the hippocampus (at day 7 and day14), like it was fixed in the group 1, is absent. However, MDA content in the somatosensory cortex (day 7) correlates with indexes of the "open field" test: with a number of grooming episodes (day7, r_s = -0,83, p<0,05) and duration of ambulation episodes in the central squares (day 14, r_s = -0,93, p<0,05). At day 14 MDA content in the somatosensory cortex inversely correlates with integral fluorescence density of NeuN+ neurons on the frontal slices of the CA1 hippocampal zone (day 7, r_s = -0,90, p<0,05), integrated fluorescence density of GFAP+astrocytes on the frontal sections of the CA1 hippocampal zone (day 14, r_s = -0,90, p<0,05), SUB-G0G1 regions on DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content < 2s in the somatosensory cortex (r_s = -0,90, p<0,05).

In rats of the group 2 SOD activity in the somatosensory cortex (day 7) doesn't inversely correlate with the number of intact nuclei in the somatosensory cortex (like it was in the group 1), but it has a correlation dependence with a number of damaged nuclei (having karyopyknosis, karyorrhexis) in the hippocampus (day 14, r_s = -0,89, p<0,05) and with the with indexes of the "open field" test (see tabl.2).

At day 14 SOD activity in the cortex of this area manifests a direct correlation link with integral fluorescence density of NeuN+ neurons on the frontal slices of the CA1 hippocampal zone (day 7, r_s = 0,90, p<0,05), integral fluorescence density of GFAP+astrocytes on the frontal sections of the CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05), SUB-G0G1 areas on DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content < 2s in the somatosensory cortex, r_s = 0,90, p<0,05).

Lactate content in the somatosensory cortex and hippocampus of rats of the group 2 correlates with indexes of the "open field" test (see tabl.2), beside this lactate content in the somatosensory cortex at day 7 directly correlates with an intact nuclei number in the somatosensory cortex (day 7, r_s = 0,90, p<0,05), at day 14 – with a damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (at day 14, r_s = 0,83 p<0,05). Lactate content in the hippocampus at day 7 inversely correlates with an intact nuclei number in the somatosensory cortex (day 7, r_s = -0,90, p<0,05) and with integral density fluorescence of NeuN+ neurons on the frontal sections of the CA1 hippocampal zone (day 7, = -0,90, p<0,05). Such the dependences between the lactate content and morphological indexed in the cortex and hippocampus don't happen in rats of the group 1.

SDH activity in the somatosensory cortex of the group 2 rats correlates not only with flow cytometry results (like it takes place in the group 1), but and with the immunohistochemical and morphological indexes, in particular SDH activity in the somatosensory cortex at day 7 has a direct link to a number of neuron nuclei in 1 mm^2 of the CA1 hippocampal zone (day 14, r_s = 0,83, p<0,05), integral fluorescence density of GFAP+astrocytes on the frontal sections of the CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05), SUB-G0G1 areas on DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content < 2s in the somatosensory cortex, r_s = 0,90, p<0,05), at day 14 – to integral fluorescence density of Iba+microglia on the frontal sections of the CA1 hippocampal zone (day 7, r_s = 0,90, p<0,05). At day 14 SDH activity in the somatosensory cortex also inversely correlates with integral fluorescence density of NeuN+ neurons on the frontal slices of the CA1 hippocampal zone (experimental day 14, r_s = -0,90, p<0,05).

Nitric oxide synthase activity in rats after IR modelling also correlates with flow cytometry indexes (like it happens in the group1, see table 1, 2) and with immunohistochemical indicators and results of the "open field" test. Thus at day 7 NOS activity in the somatosensory cortex is in the direct dependence with integrated fluorescence density of NeuN+ neurons on the frontal slices of the CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05) and in the inverse dependence with integrated fluorescence density of Iba+microglia on the frontal sections of the CA1 hippocampal zone (day 7, r_s = -0,90, p<0,05). At day 14 NOS activity in the somatosensory cortex directly correlates with an intact nuclei number in the hippocampus (day 14, r_s = 0,88, p<0,05). Nitric oxide synthase activity in the hippocampus shows numerous correlation links to the indexes 0f the "open field" test (see table 2).

NADPH oxidase activity in the somatosensory cortex in rats of the group 2 at day 7 has tight link to a damaged nuclei number (karyopyknosis, karyorhexis) in the somatosensory cortex (day 14, r_s = 0,97, p<0,01) as well as NADPH oxidase activity in the hippocampus (day 7) – to damaged nuclei number (karyopyknosis, karyorhexis) in the somatosensory cortex (day 14, r_s = 0,90, p<0,05), that doesn't meet in rats of the group1. NADPH oxidase activity in the hippocampus at day 14 directly correlates with intact nuclei number in the hippocampus (day 7, r_s = 0,84, p<0,05). Unlike the animals of the group 1 rats with IR modelling don't demonstrate visible correlations with flow cytometric indexes, but NADPH oxidase activity in the hippocampus at day 7 shows a negative link to integral fluorescence density of Iba+microglia on the frontal sections of the CA1 hippocampal zone (days 7, r_s = -0,90, p<0,05) and integral fluorescence density of Iba+microglia on the frontal sections of the CA1 hippocampal zone (days 14, r_s = -0,90, p<0,05).

Rats with IR modelling unlike the sham-operated rats demonstrate numerous direct correlations between protein content in the brain tissue and immunohistochemical, morphological and functional indexes have assessed by us. In particular protein content in the somatosensory cortex and hippocampus at day 7 directly correlates with neurological deficit (according to the McGrow Stroke-index scale) (day 7, r_s = 0,83, p<0,05 for both links). Furthermore at day 7 it directly links to integrated fluorescence density of NeuN+ neurons on the frontal slices of the CA1 hippocampal zone (day 7, r_s = 0,83, p<0,05 for the protein content in the somatosensory cortex and hippocampus separately), percentage ratio of G2 + M phase to all cells of a cell cycle in the somatosensory cortex (DNA = 4s) (day 7, r_s = 0,90, p<0,05 respectively), SUB-G0G1 areas on the DNA histograms - RN1 before the G0G1 peak (which indicates the nuclei of cells with DNA content < 2s in the somatosensory cortex), r_s = 0,90, p<0,05 respectively. In addition proteins' carbonyl groups in the somatosensory cortex at day 14 after IR have a correlation dependence with a damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (day 7, r_s = 0,83, p<0,05) and with the percentage ratio of cells in the DNA synthesis phase to all cells of a cell cycle (DNA content > 2s and < 4s) in the hippocampus (day7, r_s = 0,90, p<0,05), when at day 7 proteins' carbonyl groups in the somatosensory cortex also correlate with the percentage ratio of cells in the DNA synthesis phase to all cells of a cell cycle (DNA content > 2s and < 4s) (day 7), but in the somatosensory cortex (r_s = -0,90, p<0,05), and also with integrated fluorescence density of Iba+microglia on frontal sections of the CA1 hippocampal zone (day 7, r_s = -0,90, p<0,05).

Thus, most of the correlation dependences in rats of group 2 are given in the description; others are shown in table 2.

Group 3 (the group of rats having transfusion of MSCs from the human umbilical cord jelly at a dose 10^6 cells/rat just at once after IR making). As can be seen from the table 3 the rats treated with MSCs from the human umbilical cord jelly immediately after IR show direct correlations between glucose content in the somatosensory cortex (at day 14) and an intact neurons number in the hippocampus (days 7 and 14, r_s = 0,90, p<0,05 in both cases) similar to rats of the groups 1. Besides this glucose level in the brain tissue of the somatosensory cortex and hippocampus have plural correlations with immunohistochemical indicators, indexes of the "open field" test and flow cytometry (see the table 3). We pay an attention to the positive dependence between glucose content in the somatosensory cortex (day 14) and number of neuron nuclei in 1 mm^2 of the CA1 hippocampal zone (day 7, r_s = 0,90, p<0,05).

MDA content in the somatosensory cortex (at day 7) also demonstrates a clear direct correlation link with a number of intact nuclei in the hippocampus (at day 7 and day14, r_s = 0,90, p<0,05 for both the dates) as well as at day 14 – with neurological deficit according to McCrow Stroke- index scale (day 7, r_s = 0,78, p<0,05).

SOD activity (day 14) also shows a correlation link to intact neurons number in the hippocampus (day 7 and 14, r_s = -0,90, p<0,05 for both dates). Additional links it has to the number of neuron nuclei in 1 mm^2 of the CA1 hippocampal zone (day 7, r_s = -0,90, p<0,05) and to the percentage ratio of cells in the DNA synthesis phase to all cells

of a cell cycle (DNA content > 2s and < 4s) in both the hippocampus and somatosensory cortex (day7, r_s = -0,90, p<0,05).

When we estimate lactate content in the brain tissue a direct correlation between it in the hippocampus (at days 7 and 14) and a number of neuron nuclei in 1 mm^2 of the CA1 hippocampal zone (day 14) rushes in eyes (r_s = 0,90, p<0,05), whereas lactate content in the somatosensory cortex (day 14) inversely correlates with a number of intact nuclei in the somatosensory cortex (day 7, r_s = -0,90, p<0,05). There are numerous interrelations between lactate in the brain tissue and indexes of the "open field" test (see the table 3).

SDH activity in the somatosensory cortex (day 7) positively correlates with a number of damaged nuclei (karyopyknosis, karyorrhexis) in the somatosensory cortex (day 7, r_s = 0,90, p<0,05). Besides this it both directly and indirectly correlates with different indicators of the "open field" test (in particular, at day 7 - with latent period duration of grooming (day 7, r_s = -0,82, p<0,05), latent period duration of ambulation through the peripheral squares (day 7, r_s = -0,76, p<0,05), at day 14 – with duration of ambulation episodes through the peripheral squares (day 14, r_s = 0,89, p<0,05), latent period duration of clumbing (day 14, r_s = 0,96, p<0,01). At day 14 SDH activity shows an inverse interrelation to integral fluorescence density of Iba+microglia on the frontal slices of CA1 hippocampal zone (day 14, r_s = -0,90, p<0,05) and SUB-G0G1 areas on the DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content < 2s in the hippocampus (r_s = -0,90, p<0,05).

At day 14 (it corresponds to the recovery period of the acute cerebral ischemia) nitric oxide synthase activity in the hippocampus correlates inversely with neurological deficit according to McCrow Stroke- index scale (day 7, r_s = -0,78, p<0,05) and directly – with a number and duration of ambulation episodes through the central squares (day 7, r_s = 0,84 and r_s = 0,86 respectively, p<0,05), when oxide synthase activity in the somatosensory cortex – also directly with a number of ambulation episodes through the central squares (day 7, r_s = 0,84, p<0,05) and duration of ambulation episodes through the peripheral squares (day 7, r_s = 0,86, p<0,05).

NADPH oxidase activity in rats of the group 3 demonstrates promiscuous correlations with indexes of the "open field" test (see the tabl.3), but doesn't show a link to a damaged nuclei number (karyopyknosis, karyorhexis) in the brain tissue (like it was in rats of the group 2) or flow cytometric indicators (as in the group 1).

A negative relationship between protein content in the somatosensory cortex (at day 7) and neurological deficit according to McCrow Stroke - index scale (day 7) was found (r_s = -0,90, p<0,05). At day 14 protein content in the somatosensory cortex positively correlates with integral fluorescence density of RECA-1 positive blood vessels in the CA1 hippocampal zone (day 7, r_s = 0,90, p<0,05), when in the hippocampus (day 14) it also demonstrates a direct connection to fluorescence density of RECA-1 positive blood vessels in the CA1 hippocampal zone (day 7, r_s = 0,90, p<0,05). Additionally, protein content in the both cortex and hippocampus has a link to indexes of the "open field" test (see the table 3).

Proteins' carbonyl groups detected in the somatosensory cortex (day 7) are also in the positive relationship with indexes of the "open field" test (with a number of ambulation episodes through the peripheral squares (day 7, r_s = 0,84, p<0,05), with a number and duration of clumbing episodes (day 7, r_s = 0,89 and r_s = 0,93, p<0,05)). Moreover proteins' carbonyl groups correlate at the similar way with a percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the hippocampus (DNA content = 2s) (day 7, r_s = 0,90, p<0,05) and with integral fluorescence density of NeuN+ neurons on the frontal sections of the CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05).

Pyruvate content in the somatosensory cortex (day 7) also directly correlates with integral fluorescence density of NeuN+ neurons on the frontal sections of the CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05) and oppositely – with a number of damaged nuclei (karyopyknosis, karyorrhexis) in the somatosensory cortex (day 14, r_s = -0,90, p<0,05). Slightly lower (at day 14) pyruvate content in the somatosensory cortex has a connection to a percentage ratio of cells being in the G0G1 phase to all cells of the cell cycle in the hippocampus (DNA content = 2s) (day 7, r_s = -0,90, p<0,05). Also pyruvate accumulated in the frontal cortex changes motor activity of rats (in particular, at day 7 it has a correlation with such the indicators of the "open field" test – a number of ambulation episodes through the peripheral squares (day 7, r_s = 0,93, p<0,01) and through the central squares (day 14, r_s = 0,78, p<0,05), at day 14 – with a number of ambulation episodes through the peripheral squares (day 14, r_s = -0,78, p<0,05), a latent period duration of ambulation through the central squares (day 7, r_s = -0,78, p<0,05) and a number of grooming episodes (day 7, r_s = -0,82, p<0,05).

Ratio of lactate/pyruvate content in the somatosensory cortex (day 7) shows a direct correlation link to a number of damaged nuclei (karyopyknosis, karyorrhexis) in the somatosensory cortex (day 14, r_s = 0,90, p<0,05), integral fluorescence density of Iba+microglia on the frontal slices of CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05), and SUB-G0G1 areas on the DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content < 2s in the hippocampus (r_s = 0,90, p<0,05). At day 14 this ratio also correlates with a percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the hippocampus (DNA content = 2s) (day 7, r_s = 0,90, p<0,05) and integral fluorescence density of NeuN+ neurons on the frontal sections of the CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05).

Discussion

When analyzing correlations between morphological, biochemical, functional and immunohistochemical indicators in a brain tissue of the rat hippocampus and somatosensory cortex (regardless of whether there was brain ischemia or not) a positive correlation link was fixed up between glucose content in these brain areas and a number of intact neural nuclei (such the dependence demonstrates a fall in the undamaged neurons number in the designated brain departments with a decrease in glucose content), and a negative dependence between glucose content in these brain areas and a number of affected neurons. As is known, glucose is the main and, in fact, the most important source of energy for brain neurons,[11] when its content decreases, energy starvation of nerve cells occurs, which is manifested by a change in the behavior and motor activity of animals (changes in the "open field" test indicators are a confirmation of this, thus, changes of a latent period duration of grooming, clumbing, ambulations through the central and peripheral squares). The results of the flow cytometric and immunohistochemical investigations also lead to an opinion about a close connection between the level of glucose in brain tissue and the state of neurons. A direct correlation between the level of glucose in the cortex of the somatosensory area (day 7) and the number of damaged nuclei in the hippocampus of group 2 rats (r_s = 0,89, p<0,05) appears to be dissonant. However, it can be assumed that under conditions of ischemia with a lack of oxygen, glucose does not metabolize adequately, lactate and fats are used as an energy source,

neuronal death occurs, and the glucose level does not decrease, but perhaps relatively increases.

Lactate content in the brain tissue also has numerous correlations with the "open field" test indicators (both the content of lactate in the hippocampus and the content of lactate in the somatosensory cortex). It has been established that lactate is formed from pyruvate when it is regenerated under conditions of oxygen deficiency to ensure the further course of glycolysis (i.e., When pyruvate cannot enter the tricarboxylic acid cycle).[12] Lactate accumulation leads to the acidosis development and deterioration of metabolic processes in nerve cells. In the subacute period of brain ischemia (rats of group 2, day 7), when extracellular oedema is observed, apoptosis occurs, enzymatic imbalance in the brain tissue happens, a direct correlation is observed between the lactate content in the cortex of the somatosensory area and the number of undamaged neural nuclei in the somatosensory cortex (r_s=0,90, p<0,05). In addition, there is an inverse relationship between the lactate content in the hippocampus and the number of intact nuclei in the somatosensory cortex (on day 7, r_s = -0,90, p<0,05). On day 14 after IR, when recovery processes are actively taking place, swelling and energy deficit in the brain tissue decrease, lactate content in the hippocampus directly correlates with the number of damaged neuronal nuclei in the hippocampus (r_s=0,83, p<0,05).

When human umbilical Wharton's jelly MSCs are taken for therapy of acute brain ischemia (group 3 rats) in the subacute period of ischemia (day 7), the content of lactate in the somatosensory cortex is directly correlated with the number of intact nuclei of neurons in the hippocampus (days 7 and 14, r_s = 0,90, p<0,05, respectively, in both cases). At the same time (day 7), lactate content in the hippocampus directly correlates with the number of neuron nuclei in 1 mm^2 of the C1 hippocampal zone (day 14, r_s = 0,90, p<0,05). The same correlation dependence occurs on day 14 (i.e. in the recovery period after IR) - lactate content in the hippocampus directly correlates with the number of neuron nuclei in 1 mm^2 of the C1 hippocampal zone (day 14, r_s = 0,90, p<0,05).

It should be emphasized that with brain ischemia a ratio of lactate/pyruvate content in the somatosensory cortex directly correlates with the number of affected neuron nuclei (which have karyopyknosis or karyorrhexis) in the somatosensory cortex. In group 2, such the dependence is observed on the 14th day after IR (r_s = 0,90, p<0,05), in group 3 - on the 7th day (r_s = 0,90, p<0,05).

With brain ischemia, there is a change in the proteins level and composition in the brain tissue, the content of proteins' carbonyl groups of increases.[13] On day 7 after IR, there is a direct correlation between the protein content in the somatosensory area and neurological deficit (according to the McGrow Stroke-index scale) (day 7, r_s=0,83, p<0,05) and between hippocampal protein content and neurological deficit (according to the McGrow Stroke-index scale) (day 7, r_s=0,83, p<0,05). When using human umbilical Wharton's jelly MSCs (for the ischemia treatment), the indicated correlation dependences are also observed, but they are inverse in nature (r_s=-0,90, p<0,05). Beside this correlation connections between MDA content in the somatosensory cortex (day 14) and neurological deficit (r_s=0,78, p<0,05) appear as well as between NADPH oxidase activity in the somatosensory cortex (at day 14) and neurological deficit (r_s=-0,84, p<0,05), between NOS activity in the somatosensory cortex (day 14) and neurological deficit (r_s=-0,78, p<0,05), between NOS activity in the hippocampus and neurological deficit (r_s=-0,78, p<0,05).

In relation to enzyme activity: in the rat group without cerebral ischemia (group 1, sham-operated animals with an intact brain), a positive correlation between NOS activity in the cortex of the somatosensory area (and the hippocampus also) and the percentage ratio of G0G1 phase cells to all cells of a cell cycle in the somatosensory cortex (DNA content =2s) (day 7, r_s=0,97, p<0,01 in both cases) and a negative correlation link between NOS activity in the hippocampus and percentage ratio of the G2 + M phase cells to all cells of the cell cycle in the somatosensory cortex (DNA =4s) (day 7, r_s=-0,97, p<0,01) draw attention to themselves. Since this enzyme is responsible for the nitrogen (II) oxide synthesis (NO) from α-arginine, oxygen and NADPH, and the formed NO is important for neurotransmission, immune response formation, vascular tone control, increasing the NOS activity directly affects the normal neurons development and their number. The same relationship is observed between SDH activity in the somatosensory cortex and the percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the somatosensory cortex (DNA content = 2s) (day 7, r_s=0,90, p<0,05). SDH participates in the tricarboxylic acid cycle and the respiratory chain of electron transport, catalyzing succinate oxidation to fumarate and converting ubiquinone to ubiquinol. Thanks to participation in the energy formation in the mitochondria of cells, it is able to change cellular sensitivity to oxygen. At the same time, SDH activity determined in the cortex is inversely correlated with the percentage ratio of the DNA synthesis phase to all cells of the cell cycle in the hippocampus (DNA content > 2s and < 4s) (day 7, r_s=-0,90, <0,05) and with the percentage ratio of the cells being in the G2 + M phase to all cells of a cell cycle in the somatosensory cortex (DNA =4s) (day 7, r_s = -0,90, p<0,05), which also indicates a significant effect of this enzyme on neurogenesis. SOD is a key enzyme in the human antioxidant defense system, as it catalyzes the dismutation of oxygen superoxide and hydrogen peroxide, protects the body from highly toxic oxygen radicals. Its activity in the sham-operated animals is inversely correlated with the number of intact neuron nuclei (r_s =-0,90, p<0,05).

In the case of brain ischemia (rats of the group 2, having IR), the relationships between NOS activity and flowmetry indicators change slightly. At day 7 after IR modelling an inverse correlation between NOS activity in the hippocampus and the percentage ratio of the cells in the DNA synthesis phase to all cells of a cell cycle (DNA content > 2s and < 4s) (day 7, r_s = -0,90, p<0,05), at day 14 – between NOS activity in the hippocampus and SUB-G0G1 areas on DNA histograms – RN1 before the G0G1 peak (which indicates cell nuclei with DNA content < 2s) in the hippocampus (r_s = -0,90, p<0,05), while a correlation link between SDH activity in the somatosensory cortex and the percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the somatosensory cortex (DNA content = 2s) is absent. In addition, NOS activity in the somatosensory cortex (day 7) correlates with the results of immune histochemical analysis (with integrated fluorescence density of Iba+microglia on the frontal sections of the CA1 hippocampal zone (day 7, r_s=-0,90, p<0,05). Likewise, SDH activity correlates with indicators of immune-histochemical analysis (thus, SDH activity in the somatosensory cortex at day 7 after IR directly correlates with integral fluorescence density of GFAP+astrocytes on the frontal sections of the CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05), at day 14 - with integral fluorescence density of Iba+microglia on the frontal sections of the CA1 hippocampal zone (day 7, r_s = 0,90, p<0,05), at day 14 SDH activity in the somatosensory cortex has a negative correlation dependence with integral fluorescence density of NeuN+ neurons on the frontal slices of the CA1 hippocampal zone (experimental day 14, r_s = -0,90, p<0,05). Correlation between SDH activity recorded in healthy animals and a percentage ratio of cells being in the G0G1 phase to all cells of a cell cycle in the somatosensory cortex (DNA content = 2s) is absent, however it appears between SDH activity

in the somatosensory cortex (day 7) and SUB-G0G1 areas on DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content < 2s in the somatosensory cortex (r_s = 0,90, p<0,05) and between SDH activity in the somatosensory cortex (day 7) and the number of neuron nuclei in 1 mm^2 of the CA1 hippocampal zone (day 14, r_s = 0,83, p<0,05).

SOD activity in the somatosensory cortex (day 14) correlates with the results of immunohistochemical analysis in a similar way (compared with SDH activity), as well as SOD activity in the somatosensory cortex (day 7) has a link to the number of damaged nuclei (with karyopyknosis, karyorrhexis) in the hippocampus (day 14, r_s = -0,89, p<0,05). In the group 2 the correlation connection between NADPH oxidase activity in the somatosensory cortex (day 7) and the damaged nuclei number (with karyopyknosis, karyorhexis) in the somatosensory cortex (day 14, r_s = 0,97, p<0,01) is striking. The same is observed in the hippocampus: NADPH oxidase activity in the hippocampus (day 7 after IR) has a link to the damaged nuclei number (karyopyknosis, karyorhexis) in the somatosensory cortex (day 14, r_s = 0,90, p<0,05).

When human umbilical cord jelly MSCs are used immediately after IR in rats, the correlation dependences between NOS activity in brain tissue and flow cytometric indicators (comparing with rats of the group 1 and 2) disappear, as and the link between NOS activity and immunohistochemical indexes, which can be seen as a positive effect of the MSCs application. However, there is a negative relationship between NOS activity in the somatosensory cortex (and hippocampus at day 14) and neurological deficit (according to McCrow Stroke-index scale, r_s = -0,78, p<0,05) and also the correlation of NOS activity in brain tissue with the "open field" test indicators – that is, with purely functional indicators (in the absence of correlation with morphological ones). Instead, SDH activity (as well as SOD activity) in rats of the group 3 rats maintain a correlation with morphological indicators in the CNS. Thus, SDH activity in the somatosensory cortex (day 14) inversely correlates with integral fluorescence density of Iba+microglia on the frontal slices of CA1 hippocampal zone (day 14, r_s = -0,90, p<0,05) and SUB-G0G1 areas on the DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content < 2s in the hippocampus (r_s = -0,90, p<0,05) and at day 7 directly – with the number of damaged nuclei (karyopyknosis, karyorrhexis) in the somatosensory cortex (r_s = 0,90, p<0,05). SOD activity in the somatosensory cortex at day 7 positively correlates with integral fluorescence density of GFAP+astrocytes on the frontal slices of CA1 hippocampal zone (day 14, r_s = 0,90, p<0,05) and at day 14 negatively – with a number of intact nuclei in the hippocampus (r_s = -0,90, p<0,05), with a number of neuron nuclei in 1 mm^2 of the CA1 hippocampal zone (day 7, r_s = -0,90, p<0,05) and with S phase of a cell cycle in the hippocampus (the percentage ratio of the DNA synthesis phase to all cells of a cell cycle (DNA content > 2s and < 4s) (r_s = -0,90, p<0,05).

By the way, numerous correlations between the indicators determined in the hippocampus and the somatosensory cortex (glucose, lactate, SDH and SOD activity, etc.) indicate a close functional interaction of the specified brain departments with each other (it's observed in all rats groups).

Conclusion

a) Biochemical indicators (in particular, lactate and glucose content, MDA, SDH, SOD and NOS activity determined in the brain tissue of the hippocampal and somatosensory cortical areas), are reliably correlated with functional indicators of the central nervous system (according to the results of the "open field" test) in rats as with and both without brain ischemia.

b) Multiplex correlations between biochemical and morphological indicators determined in the somatosensory cortex and hippocampus indicate their close functional interaction (which occurs in all rat groups).

c) After acute brain ischemia at day 7 (which corresponds to the sub-acute period of ischemia) specific direct correlation dependences between protein content in brain tissue and morphological indicators appear as well as between protein content and neurological deficit (both in the somatosensory cortex and in the hippocampus). At day 14 (the recovery period) after brain ischemia making high specific inverse correlation link takes place between MDA activity in the somatosensory cortex and morphological indicators in the hippocampus.

d) No new specific correlations emerged when human umbilical cord jelly MSCs were transfused for neuro-protection immediately after brain reperfusion beginning. But some correlational dependencies disappeared (for example, the correlation dependences between NOS activity in brain tissue and flow cytometric indicators).

Acknowledgments

None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Citation: Konovalov SV, Moroz VM, Yoltukhivskyy MV., et al. Correlation between morphological, biochemical and functional indicators in acute ischemia-reperfusion rat brain. *J Stem Cell Res Ther.* 2025;10(1):53–60. DOI: 10.15406/jsrt.2025.10.00183