ORIGINAL ARTICLES

Index of lipid peroxidation and glucose utilization in the cerebrospinal fluid in patients with cerebral infarction

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Cerebral ischemia could be observed as acute metabolic crisis, when oxygen and glucose supply is compromised and synthesis of energy is insufficient. Apart from the excitotoxicity, increased production of reactive oxygen species with consequent lipid peroxidation is also included in neuronal cell damage. Furthermore, these toxic compounds could also be produced during the process of secondary inflammation of ischemic tissue. In the early stage of ischemia, as a systemic response to acute stress, there is an increase in glucose level in cerebrospinal fluid (CSF) and peripheral blood. According to the metabolic crisis and acidosis in ischemic brain tissue we investigated index of lipid peroxidation (ILP) and glucose utilization (IGU) in CSF of 53 patients of both sexes, aged 55–70 years with cerebral infarction. Control group comprised 15 patients with sudden onset of motor defficit subjected to diagnostic lumbar radiculography and suspected on discal genesis. ILP in CSF, as the indicator and sequela of neuronal cell membranes damage, was two fold increased in the acute period of cerebral infarction and maximal values (3.5 times) were noticed 24 hours after the ischemic episode compared to controls. Besides the increase in glucose concentration in peripheral blood and CSF of patients with cerebral infarction, IGU was decreased (37%) with minimal values (32%) 24 hours after the ischemia. These changes indicate that glucose is available but cells are incapable to metabolize it. We concluded that ILP and IGU in CSF of patients with cerebral infarction could be indicators of metabolic dysfunction and neuronal cell damage. Also, these results suggest the significance of polyvalent therapy including antioxidative and antiinflammatory agents in acute phase of cerebral ischemia.

Key words: **cerebral ischemia; cerebral infarction; cerebrospinal fluid; glucose; lipid peroxidation; free radicals; oxidative stress.**

Introduction

Cerebral ischemia, in general, is physiopathologic event in which the reduced cerebral blood flow (CBF) is not sufficient to maintain intracellular metabolism (1). Oxygen and glucose supply is compromised, as well as the elimination of metabolic final products. Primary event is the functional damage of ischemic tissue that is followed by tissue destruction. It is a general opinion that brain could survive ischemic episode in duration between 5 to 10 minutes, but after that period irreversibile neuronal cell damage occurs (2).

Mechanisms that lead to the ishemic neuronal cell death include all of the known mediators which cause dam-

age of selectively vulnerable neurons: (i) excitotoxicity with the increase in free cytosolic calcium, (ii) acidosis, (iii) increased generation of free radicals, (iv) enhanced production of lipid mediators (derivatives of arachidonic acid: tromboxane A_2, prostaglandins, leukotrienes and platelet activacting factor) (3).

There is an inceased level of anaerobic glycolysis in ischemic brain tissue with consequent accumulation of lactate, CO_2 and intermediate products of anaerobic metabolism. Production of reducing equivalents is insufficient with concomitant disturbance of cell respiration and energy production. These biochemical changes lead to the acidosis of ischemic brain tissue with decreased pH values from 6.5 to 6.0 (4, 5).

Persistent metabolic crisis with insufficient synthesys of ATP induces dysfunction of membrane pumps and propagation of action potentials. Dysfunction of cell membrane allows the influx of extracellular Ca^{2+} into the cell, as well as the release of sequestrated Ca^{2+} from mitochondria, endoplasmic reticulum, and synaptosomes (6).

Increase in intracellular calcium induces activation of numerous enzymes (phospholypases, proteases, endonucleases) with consequent degradation of membrane phospholipids, proteins and DNA. Altered integrity of cell membrane induces accumulation of free fatty acids in neuronal and endothelial cells. Metabolites of free fatty acids, generated in the cascades of cyclooxygenase and lipoxygenase have the character of free radicals (7).

The most important source of reactive oxygen species during ischemia is mitochondrial respiratory chain and main place of superoxide anion ($.O_2^-$) release is complex I of electron transporters. In reaction of xantine oxidase localized in the brain capillaries there is an enhanced production of hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\cdot$), $.O_2^-$, and synglet oxygen. These toxic mediators could initiate numerous reactions with autocatalytic character (8, 9).

Novel studies investigate a phenomenon of secondary developed inflammation of ischemic brain, tissue which is very important for modulation of destructive and reparative processes. In this inflammatory process the clue mediators are inflammatory and immunomodulatory cytokines; ratio of their production determines the modulation of ischemic brain lesion. Free radicals are also the mediators of inflammation, which could be produced by activated microglia. IL-4, which activates phospholipases A_2 with consequent initiation process of membrane lipid metabolism has a very important role (10, 11).

According to the metabolic crisis and acidosis in ischemic brain tissue we have proposed that index of lipid peroxidation and glucose utilization in the cerebrospinal fluid (CSF) of patients with cerebral infarction could be the indicators of metabolic dysfunction and neuronal cell damage. Furthermore, parameters of membrane lipid peroxidation could also be observed as the mediators of secondary inflammation since during the first 24 hours microglial cells in ischemic brain tissue are activated, as a potential source of reactive oxygen species (11). The aim of this study was to investigate temporal dynamics of CSF index of lipid peroxidation and glucose utilization in acute period of cerebral infarction.

Methods

Investigation included 53 patients of both sexes with completed stoke with cerebral infarction, aged between 55–70 years. Patients were without any therapy at the moment of samples collection.

Cerebral infarction was confirmed by CT scan. Also, angiography was used for the confirmation of carotid occlusion in some patients. The degree of neurologic deficits was estimated upon the Canadian Neurological Scale (12). Average value of functional dysability was 4.56 ± 1.78. According to the duration of the period after ischemic episode patients were divided into four groups: group I – 0-6 hours (n=12), group II – 7-12 hours (n=14), group III – 13-24 hours (n=14), and group IV – more than 24 hours of post-ischemic period (n=13).

Control group included 15 patients with sudden onset of motor defficit subjected to diagnostic lumbar radiculography and suspected on discal genesis. Only patients without restraint in the CSF passage were included in this study.

Samples of CSF were collected in plastic tubes and were frozen until the appropriate biochemical analysis. Samples of peripheral blood were taken from cubital vein in the plastic tubes prepared with 18.86 x 10^{-6}g Na_2EDTA and 25 IU of heparine per ml of blood.

Concentration of glucose in CSF and peripheral blood was measured according to the method with glucose oxidase (13). Index of glucose utilization (IGU) was estimated according to the next equotion:

$$IGU = \frac{\text{glycemia} - \text{glycorrhachia}}{\text{glycemia}} \times 100$$

Index of lipid peroxidaton (ILP) was determined according to the method of Andreeva et al. 1988 after stimulation of lipid peroxidation *in vitro* with Fe-salts (0.28%) (14). Thiobarbituric acid reacts with malondyaldehyde (MDA), which originates from polyunsaturated fatty acids in the process of lipid peroxidation, and forms a colored complex. Concentration of generated MDA was measured spectrophotometrically on 533 nm.

Results

Index of lipid peroxidation in CSF was two fold increased in patients with cerebral infarction, compared to controls (Fig. 1). Follow-up of temporal dynamics of ILP in CSF of stroke patients revealed that its values were the least increased in group I (from 0–6 hrs after ischemia), but with gradual increase in the other groups with maximal values more than 24 hrs after the onset of ischemia (Fig. 1).

Concentration of glucose in CSF of patients with cerebral infarction was increased, compared to the values of control group (Fig. 2). Analysis of glycorrhachia changes in function of time did not show any significant changes among groups from "0" to more than 24 hrs after ischemia (Fig. 2).

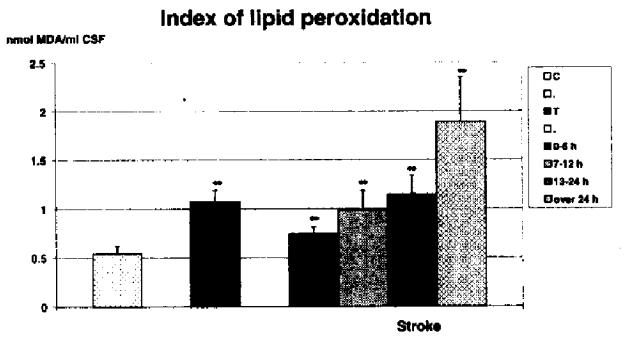

Fig. 1 – Index of lipid peroxidation in CSF of patients with cerebral infarction. Values are given as nmol MDA/ml CSF. Mean ± S.E. *- Significance to corresponding control values, (Student's t-test, $p<0.05$). Controls: 0.54 ± 0.08 nmol MDA/ ml CSF. T=average value of all patients with cerebral infarction; C=control group.

Index of glucose utilizatuon in patients with cerebral infarction was decreased, compared to controls. Temporal dynamics of IGU values progressively decreased and was maximally low 24 hours after the ischemic episode (Fig. 3).

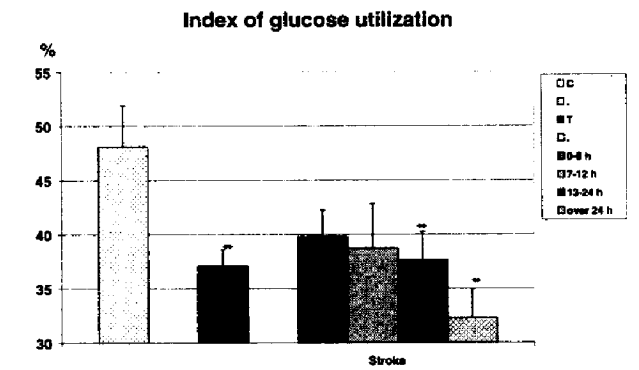

Fig. 3.– Index of glucose utilization in CSF of patients with cerebral infarction. Values are given in %. Mean ± S.E. *- Significance to corresponding control values, (Student's t-test, $p<0.05$). Controls: 48.09 ± 3.8 %. T= average value of all patients with cerebral infarction; C= control group.

Discussion

Biochemical events induced by ischemia in CNS represent physiopathologic base for ischemic neuronal cell damage, and include activation of different mediator systems. Cerebral ischemia activates cascade of free radical reactions, which lead to damage of biomolecules, disturbance of intracellular metabolism, as well as capability of adaptive response, with consequent neuronal cell death (15, 16).

Experimental models of cerebral ischemia confirmed that in an early stage of brain ischemia in the forebrain cortex and striatum of Mongolian gerbil exited the increase of superoxide anion production, as well as of other active oxygen species. These changes lead to the chain propaga-

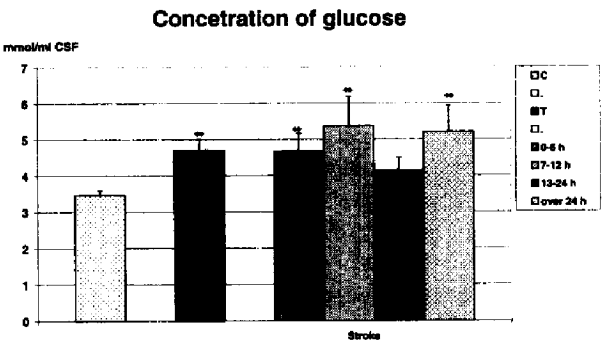

Fig. 2. – Concentration of glucose in CSF of patients with cerebral infarction. Values are given as nmol/ml CSF. Mean ± S.E. *- Significance to corresponding control values, (Student's t-test, $p<0.05$). Controls: 3.47 ± 0.12 mmol/ml CSF. T=average value of all patients with cerebral infarction; C=control group.

tion of free radical reactions, increase in superoxide anion/superoxide dismutase ratio, and decreased activity of glutathione reductase, suggesting the insufficient functions of antioxidative defense system for $.O_2^-$ and H_2O_2 detoxification, as well as for other peroxides formed in reactions of reactive oxygen species with biomolecules (17, 18).

One of the consequences of free radical reactions is lipid peroxidation, which causes dysfunction and damage of cell membranes. Increased index of lipid peroxidation in CSF of patients with cerebral infarction could indicate the activated and operative oxidative stress (Fig. 1). Furthermore, content of MDA was gradually increased from "0" to more than 24 hours after ischemia, suggesting the temporal propagation, as well as amplification of process of lipid peroxidation. Consequences of these toxic reactions are the affected functions and integrity of membranes accompanied with dysfunction of membrane enzymes, alteration of receptors presentation for neurotransmitters, as well disturbance of membrane permeability (19).

These changes of MDA in CSF could reflect biochemical events at the cellular level in CNS after the ischemic episode with possible predictive significance for clinical outcome. Also, these changes indicate the importance of administration of antioxidative therapy in acute period of ischemia to prevent damaging effects of lipid peroxidation. Since free radicals and peroxides are also mediators of inflammation, antiinflammatory therapy could be helpful in modification of ischemic brain damage. Experimental studies of brain ischemia have found that secondary developed inflammatory process in ischemic tissue include local reaction of resident brain cell (astrocytes, microglia, and neurons), as well as infiltration of peripheral inflam-

matory cells (neutrophylles and monocytes) and endothelial cell activation. Activated microglia, macrophages and leukocytes produce the increased quantity of $OH^{\cdot}$, H_2O_2 and NO. Some of these reactive species like $OH^{\cdot}$ and peroxynitrite ($ONOO^-$), which is formed in reaction of $.O_2^-$ and NO, could initiate process of lipid peroxidation (20, 21).

Increase in glucose level in CSF and peripheral blood occurs in the early stage of ischemia as the systemic response to acute stress (Fig. 2). Temporal dynamics of glycorrhachia variation in acute period of cerebral infarction was not significant (Fig. 2). During brain ischemia, in first 10–20 seconds of compromised circulation, after the utilization of residual tissue reserve, oxidative phosphorylation is interrupted. These changes lead to exhaustion of energy reserve: ATP, P-creatine, glycogen, as well as NADH and NADPH decrease, at the other hand lactate, pyruvate, NAD and NADP are in increased concentration. Tissue acidosis blocks glycolysis; glucose is present but can not be utilized (22).

Index of glucose utilization in patients with cerebral infarction was decreased compared to control group (Fig. 3). This index gradually decreased from "0" to more than 24 hours of acute ischemia, suggesting the alteration of glycolytic pathway in direction of anaerobic glycolysis. Furthermore, function of clue enzymes of glycolysis is disrupted with consequent reduction of glucose-6-phosphate and fructose-6-phosphate, and with increased content of other intermediaries (20, 22). These data indicate the dissociation between the available glucose and cell capability to metabolize it. Thus, the glucose utilization is decreased as well as the energy production, what is necessary for normal metabolism and active transport, as well as enzyme activities.

Increase of lipid peroxidation index and decrease in glucose utilization in CSF of patients with cerebral infarction indicate the significance of early diagnosis and estimation of metabolic profile neuronal cell with the aim of cellular protection from toxic free radical reactions, as well as prevention of energy inssuficiency.

Conclusion

Results obtained in this study could indicate that:

1. Cerebral infarction induces numerous physiopathologic processes in neuronal cells, which could be reflected as biochemical changes in CSF. Measurement of these biochemical changes could be significant for the early diagnosis and estimation of intensity of neuronal cell damage.

2. Index of lipid peroxidation in CSF as the indicator and sequela of neuronal cell membranes damage, was increased in acute period of cerebral infarction and maximal values were noticed 24 hours after the ischemic episode.

3. Besides the increase in glucose concentration in peripheral blood and CSF of patients with cerebral infarction, index of glucose utilization was decreased with minimal values 24 hours after the ischemia. These changes indicate that glucose is available but cells are incapable to metabolize it.

4. Also, these results suggest the significance of polyvalent therapy including antioxidative and antiinflammatory agents in acute phase of cerebral ischemia.

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The paper was received on August 2, 1999.

Апстракт

Selaković V, Jovanović MD, Jovičić A, Mršulja BJ, Maličević Ž, Ninković M, et al.
Vojnosanit Pregl 2000; 57(4): 375–379.

ИНДЕКС ЛИПИДНЕ ПЕРОКСИДАЦИЈЕ И ИСКОРИШЋАВАЊА ГЛУКОЗЕ У ЦЕРЕБРОСПИНАЛНОЈ ТЕЧНОСТИ БОЛЕСНИКА СА ИНФАРКТОМ МОЗГА

Исхемија мозга се може схватити као акутна метаболичка криза, у којој је компромитовано снабдевање мозга кисеоником и глукозом што је праћено смањеним стварањем енергије. Поред ексцитотоксичности, повећано стварање реактивних оксидативних материја са последичном пероксидацијом липида такође је укључено у настанак оштећења нервне ћелије. Даље, ове токсичне компоненте могу се стварати и у току процеса секундарне инфламације исхемичног ткива. У раном периоду исхемије, као системски одговор на акутни стрес, повећава се ниво глукозе у цереброспиналној течности (ЦСТ) и периферној крви. На основу доказа о настајању метаболичке кризе и ацидозе у исхемичном можданом ткиву испитивали смо индекс липидне пероксидације (ИЛП) и искоришћавања глукозе (ИИГ) у ЦСТ 53 болесника оба пола са инфарктом мозга. Контролна група је обухватила 15 болесника са наглим развојем моторног дефицита сумњиве дискалне генезе; подвргнутих дијагностичкој лумбалној радикулографији просечне старости од 55 до 70 година. ИЛП у ЦСТ, као индикатор и последица оштећења неуронских мембрана, два пута је повећан у акутном периоду инфаркта мозга са максималним вредностима (3,5 пута) 24 часа после епизоде исхемије у односу на контролну вредност. И поред повећања концентрације глукозе у периферној крви и ЦСТ болесника са инфарктом мозга ИИГ је снижен (37%) са максималним вредностима (32%) 24 часа након исхемије. Ове промене указују на то да је глукоза расположива, али ћелије нису у стању да је метаболишу. Закључили смо да ИЛП и ИИГ у ЦСТ болесника са инфарктом мозга могу бити показатељи метаболичке дисфункције и оштећења нервне ћелије. Такође, добијени резултати сугеришу на велику важност поливалентне терапије, укључујући антиоксидавне и антиинфламаторне супстанце у акутној фази исхемије мозга.

Кључне речи: **mozak, veliki, ishemija; mozak, veliki, infarkt; cerebrospinalna tečnost; glukoza; lipidi, peroksidacija; slobodni radikali; stres, oksidativni.**