DOI 10.1007/s10517-017-3764-4
Bulletin of Experimental Biology and Medicine, Vol. 163, No. 2, June, 2017

PATHOPHYSIOLOGY

Assessment of Oxidative Status of the Brain and Blood Plasma in Rats with Modeled Focal Cerebral Ischemia/Reperfusion Injury

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 2, pp. 156-159, February, 2017
Original article submitted November 30, 2016

Parameters of the oxidative status of the brain and blood plasma were measured in rats 24 h after 1-h focal cerebral ischemia. In the brain of rats exposed to cerebral ischemia, activities of superoxide dismutase and catalase were elevated. Ischemia reduced the total antioxidant activity of the brain and the levels of malonic dialdehyde and protein carbonyl derivatives. In the blood plasma of experimental rats, superoxide dismutase activity and malonic dialdehyde level increased and total antioxidant activity decreased, *i.e.* the shifts were similar to those in the brain. The ischemia-induced changes in the brain and blood were not always co-directed.

Key Words: *focal cerebral ischemia; oxidative stress; brain; blood plasma*

Oxidative stress is one of major pathophysiological mechanisms of damage to the nervous tissue in the ischemic brain and an important factor reflecting the severity of ischemic lesion [12]. There is an increasing body of evidence describing oxidative stress and its role in the damage to the nervous tissue in ischemic brain [9,10]. However, the development of oxidative stress and its temporal parameters in focal cerebral ischemia are still the avenues of intensive study [3]. Most biochemical data on oxidative stress had been obtained by examination of human blood [7], but the corresponding parameters were not examined in the brain tissue. The current paradigm assumes that the changes in blood plasma parameters reflect similar processes in the brain, although there are few studies, which simultaneously assessed the blood and brain parameters of oxidative stress [4].

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Our aim was to assess oxidative status by activity of antioxidant defense system (ADS) enzymes, total antioxidant activity, and the levels of lipid and protein oxidation products in the brain tissue and blood plasma of rats with focal ischemia/reperfusion of the brain.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (body weight 300-330 g) maintained on 20 g/day semisynthetic diet (the food was similar to commercial product AIN-93M) with a 12-hour day-night cycle, vivarium temperature 25±2°C, and water *ad libitum*. The rats were randomized as follows: group 1, of sham-operated animals (n=20) subjected to all surgical manipulations except for occlusion of the middle cerebral artery; group 2, ischemic rats (n=12) used to assess the biochemical parameters; and group 3, ischemic rats (n=12) employed to assess the area of cerebral lesion. The focal cerebral ischemia was modeled by 1-h intra-

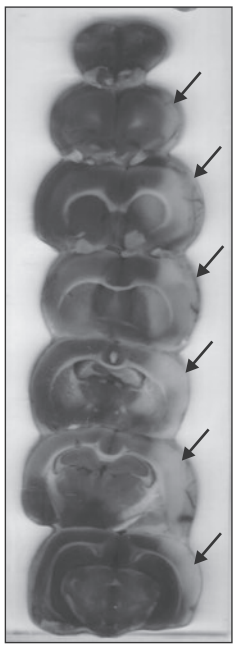

Fig. 1. Cerebral sections cut after occlusion of the middle cerebral artery. Ischemic focus is shown with arrows. 2,3,5-Triphenyltetrazolium chloride staining.

luminal occlusion of the middle cerebral artery with a silicon monofilament [15].

In 24 h after surgery, the rats were decapitated, the brain was excised and immediately frozen for subsequent biochemical assay and assessment of the size of ischemic focus. The frozen ischemic hemisphere was homogenized in cold PBS (0.1 M; pH 7.4; 10 ml buffer per 1 g brain tissue) in a Potter—Elvehjem homogenizer for 1 min at 900 rpm. Prior to homogenization, the coronal section was cut from the brain of ischemic rats at the level of hypophysis for morphological control of ischemia. The brains without necrotic focus were discarded from examination.

The blood was collected into heparinized (1000 U/ml) glass tubes and centrifuged at 3000 rpm for 20 min, thereupon the blood plasma was frozen at -80°C for later examination.

The content of malonic dialdehyde (MDA) and protein carbonyl derivatives [5], total antioxidant activity (employing Ferric Reducing Antioxidant Power detection kit), total superoxide dismutase (SOD) activity, and glutathione peroxidase (GPx) activity in the brain and blood plasma were assayed spectrophotometrically [1]. In addition, the following parameters of oxidative stress were assessed by Fe^{2+}-induced chemiluminescence [8]: the level of preformed lipid hydroperoxides (h, mV) and oxidation resistance (latency τ, sec), which assesses activity of endogenous ADS. The cerebral homogenates were used to measure activity of catalase and glutathione S-transferase (GST) [1].

To assess ischemic focus area, the frozen brain was cut into 1-2-mm coronal sections, which were stained for 10 min in 2,3,5-triphenyltetrazolium chloride (2%) dissolved in PBS (0.1 M; pH 7.4) at 37°C. The sections were bilaterally scanned at 600 dpi (Fig. 1) and analyzed with an ImageJ software. To correct the data for brain edema and scatter in brain size, the area of necrotic damage was scaled in percentage of that of the contralateral hemisphere.

The data were analyzed statistically using Statistica 12.0 and Microsoft Excel software. Significance was assessed with Mann—Whitney test at $p \leq 0.05$. The results are summarized as $m \pm SEM$.

RESULTS

The damaged area in the sections of ischemic brain occupied 26.2±1.3% total area of the hemisphere (n=9), which fell within the range of reported data [13]. Thus, the study employed a reasonable model with admissible scattering in focal ischemia size.

The focal brain ischemia followed by a 24-h reperfusion enhanced activities of two enzymes of brain ADS: SOD by 41% and catalase by 24% (Table 1). In the brain, GPx and GST activities and the level of preformed lipid hydroperoxides did not significantly change, while MDA and the protein carbonyl derivatives decreased by 47 and 29%, respectively. At the same time, both employed methods revealed a diminished total non-enzymatic antioxidant activity in the ischemic hemisphere. Thus, the total antioxidant activity and oxidation resistance degraded by 24 and 25%, respectively.

As a rule, the studies employing parameters of oxidative status as the indices of neuroprotective effects exerted by a tested chemical agent report a drop in activity of ADS enzymes accompanied by elevation of the oxidative stress products [6,14]. In contrast, other researchers observed elevation of ADS enzymatic activity provoked by focal ischemia viewed as a response to production of a large amount of chemically reactive free radicals [2]. A hypothesis was advanced that ischemia-provoked oxidative stress is a biphasic process characterized by the first (rapid) phase, which is directly related to oxidative burst during reperfusion, and the second phase reflecting the development of inflammation [11]. Reduced content of lipid and protein oxidation products can indicate termination of the first phase of the oxidative stress. The development of oxidative stress in the brain tissue is indicated by up-regulation of SOD and catalase activities accompanied by down-regulation of total non-enzymatic antioxidant activity in this tissue.

Similar to the brain, assessment of oxidative status in blood plasma of ischemic rats revealed up-

TABLE 1. Effect of Focal Brain Ischemia on Antioxidant Parameters in Rat Brain and Blood Plasma (*m*±*SEM*)

Parameter		Control	Ischemia
GPx, nM NADPH oxidized/(min×mg protein)	brain	8.194±0.236	8.909±0.322
	plasma	10.102±0.504	8.622±0.300
SOD, activity/(min×mg protein)	brain	2.27±0.23	3.21±0.27*
	plasma	0.119±0.005	0.147±0.001*
Catalase, μmol/(min×mg protein)	brain	4.16±0.17	5.18±0.29**
GST, nmol/(min×mg protein)	brain	8.68±0.23	9.75±0.50
Total antioxidant activity, mM Fe^{2+} equivalent	brain	7.38±0.35	5.63±0.21**
	plasma	0.387±0.0025	0.441±0.02
Oxidation resistance, sec	brain	98.22±6.08	74.40±4.07*
	plasma	64.28±3.16	45.00±2.04*
Lipid hydroperoxides, mV	brain	156.12±4.30	134.39±9.19
	plasma	59.68±2.04	67.30±4.03
MDA, nmol/g (nmol/ml)	brain	197.86±8.17	104.69±7.18**
	plasma	4.58±0.11	5.26±0.12*
Protein carbonyl derivatives, nmol/mg protein	brain	12.97±1.12	9.27±1.10*
	plasma	2.57±0.09	2.66±0.15

Note. *p<0.05, **p<0.001 in comparison with the control.

regulation of SOD activity by 23% and a decrease in oxidation resistance by 30%. In contrast, elevation of plasma MDA by 14% did not reflect its change in the brain, which can result from accumulation of this stable aldehyde in the blood due to its release from the cerebral tissue. In contrast to the brain, ischemia did not change the total antioxidant activity and the levels of oxidized proteins in blood plasma. A similar study [4] showed that cerebral ischemia elevated MDA in both blood and brain; at this, MDA elevation in the blood was more pronounced than in our experiments. However, in contrast to focal ischemia used in our work, the collated study employed global ischemia of entire cerebral hemisphere by ligating the left common carotid artery for 30 min [4].

Thus, focal cerebral ischemia followed by 24-h reperfusion up-regulated activities of two antioxidant enzymes (SOD and catalase), down-regulated production of oxidized lipids and proteins, and moderated the total antioxidant activity in the brain. Elevation of SOD activity and MDA content in blood plasma accompanied by a drop in oxidation resistance also attest to disturbance of oxidative status provoked by focal cerebral ischemia. However, the plasma parameters did not closely correlated with the changes in the brain, so the blood plasma indices cannot reliably assess the level of oxidative stress in the brain subjected to focal ischemia and reperfusion.

We thank Dr. L.V. Kravchenko, an out-of-staff member of Federal Research Center of Nutrition and Biotechnology, for his contribution to experimental work and editing the manuscript.

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