Correction of Consequences of Postischemic Reperfusion Brain Damages with Cytoflavin

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Cytoflavin normalized energy metabolism, decreased the intensity of lipid peroxidation, and restored activity of the antioxidant system in rat brain during postischemic reperfusion. Cerebroprotective effect of cytoflavin was similar to that of piracetam.

Key Words: *cytoflavin; piracetam; ischemia; postischemic reperfusion*

Hypoxia plays the major role in the pathogenesis of acute ischemic damages to cerebral blood flow. Hypoxia triggers the cascade of metabolic transformations modulating the intensity of energy metabolism and lipid peroxidation (LPO) [1,8]. Therefore, the therapy of acute cerebral ischemia should include not only correction of cerebral blood flow with vasoactive, fibrinolytic, and antiaggregant agents, but also normalization of energy metabolism and stabilization of cell membranes and subcellular structures in nerve cells.

Here we studied effects of cytoflavin on brain metabolism during postischemic reperfusion damage. Cytoflavin (Polysan) is a complex preparation containing succinic acid, purine nucleoside (riboxine), nicotinamide, riboflavin mononucleotide, and N-methyl-D-glucamine (solubilizer).

MATERIALS AND METHODS

Experiments were performed on 100 male outbred albino rats weighing 180-200 g (Rappolovo nursery). Ischemic brain damage was induced by 90-min occlusion of the common carotid arteries followed by reperfusion. The effect of cytoflavin (1.5 ml/kg) on postischemic brain damage was examined 24 and 72 h after reperfusion. The effects of cytoflavin were compared with those of piracetam (100 mg/kg). Control animals received an equivalent volume of physiological saline. The preparations were injected intraperitoneally 2 times a day. Control group comprised sham-operated rats.

Energy metabolism in brain tissue was evaluated by the content of lactate and pyruvate [15] and lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) activities [5,6]. The intensity of LPO was estimated by the concentrations of malonic dialdehyde (MDA) and conjugated dienes (CD) of unsaturated fatty acids [12,13]. The state of the antioxidant system was analyzed by superoxide dismutase (SOD) activity and content of reduced glutathione (GSH) [3,11].

The results were analyzed by Student's t test.

RESULTS

The content of lactate and LDH activity in rat brain after common carotid artery occlusion increased by 194 and 107%, and pyruvate concentration and SDH activity decreased by 54 and 51%, respectively, compared to the control (Table 1). The lactate/pyruvate ratio reflecting the intensity of aerobic or glycolytic carbohydrate metabolism increased from 8.6 to 55.4. These data indicated inhibition of aerobic and stimulation of glycolytic (emergency) mechanisms of energy formation.

The intensity of LPO increased, and the antioxidant system was suppressed in the ischemic brain. The

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contents of CD and MDA increased by 78 and 54%, and SOD activity and GSH concentration decreased by 42 and 35%, respectively. It was established that free radicals generated after LPO activation can damage membrane structures of nerve cells or cause cell death [4].

Metabolic changes in the brain of rats receiving physiological saline depended on the time after reperfusion. On day 1 after reperfusion, the lactate/pyruvate ratio increased to 64.7 indicating inhibition of aerobic and stimulation of glycolytic pathways of energy production. The contribution of aerobic pathways increased on day 3 after reperfusion: the lactate/pyruvate ratio decreased to 19.7.

On day 1 after reperfusion, the content of CD did not differ from the initial level, and MDA concentration decreased by 17% (compared to that during occlusion) indicating inhibition of LPO processes. SOD activity and the content of GSH increased by 15% and 11%, respectively.

On day 3 after reperfusion, the intensity of LPO increased. The content of MDA increased by 82% compared to that on day 1 after reperfusion. At the same time, the concentration of CD decreased by 30% probably due to their conversion into secondary LPO products. The content of GSH decreased by 36% indicating inhibition of the glutathione enzyme system.

Cytoflavin induced positive effects on energy metabolism in nervous tissues on day 1 after reperfusion. The content of pyruvate and SDH activity increased by 100 and 86%, and lactate concentration and LDH activity decreased by 47 and 40%, respectively, compared with placebo. The lactate/pyruvate ratio decreased 3.7-fold. On day 3 after reperfusion, the parameters of energy metabolism in cytoflavin-treated animals did not differ from the control.

Cytoflavin not only inhibited LPO and restored the antioxidant system activity on day 1 after reperfusion, but also prevented LPO intensification on day 3 after reperfusion.

There were no significant differences in the effects of cytoflavin and piracetam on metabolism of nervous tissue and animal survival under conditions of impaired cerebral blood flow.

Most effects of piracetam, including its antiischemic action, are associated with changes in intracellular processes (activation of aerobic and anaerobic glucose oxidation, stimulation of RNA and protein synthesis, activation of adenylate cyclase, and inhibition of LPO in mitochondria) [2]. The analysis of cytoflavin components suggests that the antiischemic activity of this preparation is related to its effects on metabolic processes in nerve cells. Succinic acid and riboxine are neurometabolic stimulators activating RNA and protein synthesis and improving the energy state of neurons [7,9]. Succinic acid is a potent mitochon-

TABLE 1. Effects of Cytoflavin and Piracetam on Ischemic Brain Metabolism ($M \pm m$, n=10)

Parameter	Control	90-min ischemia	Reperfusion, day 1/day 3		
			placebo	cytoflavin	piracetam
Pyruvate, μM/g	0.24±0.01	0.11±0.01*	0.08±0.01*/0.17±0.01*	0.16±0.01+/0.26±0.11+	0.18±0.02+/0.25±0.01+
Lactate, μM/g	2.07±0.04	6.09±0.07*	5.18±0.27*/3.35±0.11*	2.77±0.08+/1.99±0.05+	2.51±0.16+/2.31±0.02+
Lactate/pyruvate	8.6	55.4	64.7/19.7	17.3/7.6	13.9/9.24
LDH, μM NADH/min/mg protein	1.08±0.08	2.24±0.14*	2.01±0.04*/1.16±0.04	1.21±0.09+/1.12±0.07	1.60±0.06+/0.96±0.05+
SDH, nM succinate/min/mg protein	8.44±0.14	4.16±0.15*	5.39±0.19*/7.92±0.24	10.05±0.49+/7.95±0.19	6.33±0.19+/8.03±0.12
CD, μM/g	21.65±0.35	38.51±0.69*	21.00±2.08/14.82±2.07*	21.96±1.69/17.19±1.00	19.11±1.89/17.65±0.38
MDA, μM/g	6.58±0.16	10.16±0.22*	8.49±0.16*/15.46±0.19*	7.28±0.28+/9.26±0.25+	9.53±0.14+/10.26±0.34+
SOD, rel. units/mg protein	3.09±0.07	1.81±0.04*	2.09±0.12*/3.10±0.11	2.73±0.15+/3.31±0.28	3.46±0.32+/2.85±0.11
GSH, μM/g	42.42±0.68	27.73±0.03*	30.73±0.29*/19.61±0.74*	40.49±0.54+/36.5±0.42+	38.35±0.32+/28.90±1.23+

Note. *$p<0.05$ compared to the control and +$p<0.05$ compared to the placebo.

drial antioxidant [7], nicotinamide stimulates biosynthesis of NAD coenzymes and ATP formation [10], and riboflavin possesses considerable antihypoxic properties [14].

Our results indicate that cytoflavin protects the brain during postischemic reperfusion due to its effects on metabolic processes in the central nervous system and, therefore, it would be appropriate for use in medical practice for protection of the brain against circulatory disturbances.

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