Antioxidant Effects of Bemitil during Acute Cerebral Hypoxia

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Bemitil (25 mg/kg) prevented activation of lipid peroxidation and inhibition of the antioxidant system in rat brain during acute hypoxic hypoxia.

Key Words: *bemitil; hypoxia; lipid peroxidation; antioxidant system*

Intensive oxidative processes in the brain, low carbohydrate reserves, and high content of lipids (particularly, polyunsaturated fatty acids) promote free radical reactions during acute hypoxia. Overproduction of reactive oxygen species in the central nervous system determines high activity of the antioxidant systems in the brain. However, activities of the key antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase in the brain are lower than in other organs. It remains unclear whether generation of lipid peroxidation (LPO) products or decrease in the activity of antioxidant systems plays the primary role in oxidative stress during brain hypoxia. These processes are realized via disintegration of metabolic reactions and energy exchange accompanying oxygen deficiency. In light of this, medicinal preparations with metabolic activity are more preferable for brain protection from acute hypoxia. Bemitil was synthesized at the Department of Pharmacology (Military Medical Academy). The preparation maintains locomotor activity and physical capacity under extreme conditions, including overwork, heavy and repeated exercises, overheat, and oxygen deficiency. Bemitil normalizes intracellular metabolic processes via the activation of RNA and protein synthesis [1]. Bemitil (bemactor, ICN, Oktyabr) is widely used under normal conditions and for the therapy of progressive muscular dystrophy, myocardial ischemia and infarction, and ischemic insult [4].

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Here we studied the antioxidant effect of bemitil on rat brain during acute hypoxic hypoxia.

MATERIALS AND METHODS

Experiments were performed on male rats weighing 160-180 g. Acute hypoxic hypoxia was produced in a pressure chamber. The rats were maintained to a simulated altitude of 8000 m above sea level for 30 min (50 m/sec). Bemitil was injected intraperitoneally in an optimal effective dose of 25 mg/kg 30 min before hypoxia. Control animals received 0.9% NaCl. The rats were decapitated immediately after hypoxia.

Brain samples were frozen in liquid nitrogen. The contents of conjugated dienes [5] and ketotrienes [6], malonic dialdehyde (MDA), protein SH groups [5], and reduced glutathione (GSH) and activities of glutathione reductase [3], glutathione transferase [7], glutathione peroxidase (GSH-Px) [9], and SOD were measured [2]. Enzyme activities were calculated per protein content [8].

The results were analyzed by Student's t test.

RESULTS

Acute hypoxia was accompanied by accumulation of LPO products in the brain. The concentrations of conjugated dienes, ketotrienes, and MDA increased by 13, 21, and 89%, respectively ($p<0.05$, Table 1). The contents of GSH and free SH groups decreased by 27 and 15%, respectively. These changes were accompanied by a decrease in SOD activity (by 26%) and activity of other antioxidant enzymes regulating the ratio between oxidized and reduced

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TABLE 1. Effects of Bemitil on LPO and Activity of Antioxidant Enzymes in Rat Brain after Acute Hypoxia ($M \pm m$, n=8)

Parameter	Intact animals	Hypoxia	
		no correction (control)	bemitil
Conjugated dienes, nmol/g tissue	18.33±0.83	20.75±0.66*	16.25±0.49*+
MDA, nmol/g tissue	8.85±0.63	16.69±0.24*	9.25±0.64+
SOD, U/mg protein	2.63±0.03	2.20±0.05*	2.24±0.06*+
SH groups, μmol/g tissue	3.47±0.07	2.96±0.10*	3.49±0.07+
GSH, μmol/g tissue	31.57±0.82	23.10±1.23*	28.36±1.02*+
Glutathione reductase, nmol NADPH/min/mg protein	22.97±0.35	19.65±0.53*	26.72±0.88*+
GSH-Px, nmol HADPH/min/g protein	6.98±0.06	5.84±0.28*	7.98±0.25*+
Glutathione transferase, nmol DCNB/min/mg protein	61.06±0.64	57.50±1.12*	59.81±2.97

Note. $p<0.05$: *compared to intact animals; +compared to the control. DCNB: dichloronitrobenzoic acid.

glutathione. It should be noted that glutathione system deficiency during acute hypoxia can lead to sharp intensification of free radical oxidation, membrane destruction, and neuronal death. Activity of glutathione reductase that replenish GSH pool in rat brain decreased by 14% during acute hypoxia. Activity of GSH-Px, which is involved (together with glutathione reductase) in the glutathione redox cycle, decreased by 26% ($p<0.05$). Glutathione transferase activity in the brain did not change during acute hypoxia. Therefore, the antioxidant system in nerve cell nuclei remained unchanged.

Thus, acute hypoxia was followed by accumulation of primary and secondary LPO products and inhibition of the antioxidant system in rat brain.

Administration of bemitil 30 min before hypoxia prevented accumulation of conjugated dienes, ketotrienes, and MDA in rat brain by 22, 67, and 45%, respectively ($p<0.05$, Table 1). Bemitil increased the content of protein SH groups in rat brain by 18%. The increase in GSH concentration induced by bemitil (by 23%) was probably related to the activation of glutathione reductase. In rats receiving bemitil glutathione reductase activity surpassed that in control and intact animals (by 36 and 16%, respectively, $p<0.05$, Table 1). These data show that bemitil restores the content of thiol components, which maintains cell membrane integrity, membrane proteins, and enzyme activity.

Bemitil normalized activity of antioxidant enzymes playing a key role in the glutathione system. In rats receiving bemitil GSH-Px activity was higher than in control and intact animals (by 37 and 14%, respectively). Glutathione transferase activity in bemitil-treated rats did not differ from that in intact animals. Probably, bemitil-induced normalization of these synergistically acting enzymes provides effective and multilevel protection of neurons from reactive oxygen species and LPO products. The activities of glutathione reductase, which is involved in GSH resynthesis, and SOD increased by 36 and 10%, respectively ($p<0.05$).

Our findings suggest that bemitil produces a pronounced antioxidant effect on rat brain during acute hypoxic hypoxia. Administration of bemitil in a single dose of 25 mg/kg 30 min before hypoxia prevents LPO intensification and inhibition of antioxidant enzymes and activates GSH-Px and glutathione reductase in rat brain.

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