Cerebroprotective Effect of Combined Treatment with Pyrazidol and Bemitil in Craniocerebral Trauma

I. V. Zarubina, N. A. Kuritsyna, and P. D. Shabanov

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Monotherapy of consequences of craniocerebral trauma with pyrazidol (1 mg/kg) produced an anxiolytic effect in animals highly resistant to hypoxia and activating effect on low resistant animals. Treatment with bemitil in a dose of 25 mg/kg produced a cerebroprotective effect and normalized individual behavioral characteristics, parameters of energy metabolism, and state of the antioxidant system in the brain of highly and low resistant rats. The effect of bemitil was most pronounced in highly resistant animals. During combined treatment, pyrazidol and bemitil had an additive effect in animals of both groups. They normalized behavioral reactions and prevented the development of metabolic disturbances in the brain.

Key Words: *craniocerebral trauma; bemitil; pyrazidol; behavior; metabolism*

The postcommotional period of craniocerebral trauma (CCT) is characterized by long-term rehabilitation of patients. The pathogenesis of CCT includes neurodynamic and metabolic disorders of the central nervous system that are primarily related to hypoxic injury of the nervous tissue and results in the development of posthypoxic encephalopathy. Psychopathological syndrome is a common delayed consequence of CCT manifested in asthenic, neurotic, and neurosis-like disorders [10]. The severity of psychovegetative syndrome and emotional-and-intellectual disturbances progressively increases after CCT. Pathological changes in the brain develop in the delayed period after CCT and are similar to those observed in depressive patients [11].

The general principle of pharmacotherapy for CCT is combined treatment with considering pathogenetic characteristics of the disease. The standard therapy for posttraumatic disorders should include pharmacological preparations with nootropic and antihypoxic activity. To avoid overuse of medications for combined treatment of patients with CCT, it is necessary to synthesize new preparations with specific pharmacological activity. Antihypoxant bemitil (2-ethylthio-benzimidazole hydrobromide) possesses nootropic and mild psychostimulant activity and fully conforms these requirements [1,7].

Here we studied the cerebroprotective effect of individual and combined treatment with the antihypoxant bemitil and antidepressant pyrazidol in animals with CCT characterized by different resistance to hypoxia.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 160-180 g. Each group included 8-10 animals. The animals were divided into groups by the resistance to acute hypoxia. Hypoxia was produced in an altitude chamber. The rats were "elevated" to a height of 12,000 m (50 m/sec) and were maintained under these conditions until the appearance of agonal breathing. The animals that survived for 5-10 and more than 10 min were considered to be low resistant (LR) and highly resistant (HR), respectively. Mild closed CCT was produced 24 h after hypoxia. The weight of 64 g fell freely from a hollow tube (height 80 cm, diameter 1.3 cm) to the parietal region of the head [8]. The head was fixed on a soft pad to prevent

Department of Pharmacology, S. M. Kirov Military Medical Academy, Russian Ministry of Defense, St. Petersburg

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jaw fracture. The animals with depressed fracture of the parietal lobe died over the first minutes after CCT (not more than 5% rats). The brains from died animals were not examined.

The rats were divided into groups. Group 1 animals received intraperitoneal injections of bemitil in a dose of 25 mg/kg (ICN-Oktyabr') for 20 days. Pyrazidol in a dose of 1 mg/kg (Farmakon) was injected intraperitoneally to group 2 rats. Combined treatment with 1 mg/kg pyrazidol and 25 mg/kg bemitil was applied to group 3 animals. Control rats received an equivalent volume of physiological saline.

The conclusion about the positive effect of drugs on animals with CCT was based on survival rate, behavioral reactions, and biochemical assays. The physiological reaction of rats to CCT was studied in the open field and elevated plus-maze tests. We recorded orientation-and-exploratory, emotional, stereotypic, and locomotor activity of animals. The state of energy metabolism in the brain was determined by the contents of creatine phosphate [6], ATP [4], lactic acid, and pyruvic acid [6] in brain tissues pre-frozen in liquid nitrogen. The intensity of lipid peroxidation (LPO) was estimated by the concentrations of conjugated dienes and malonic dialdehyde (MDA) [9]. The state of the antioxidant system was evaluated by the amount of reduced glutathione [6] and superoxide dismutase (SOD) activity [2]. Enzyme activity was calculated per protein content in samples measured by the method of Lowry [6].

The results were analyzed by Student's t test.

RESULTS

The rats phenotypically differing in the resistance to hypoxia before CCT exhibited various behavioral reactions. HR animals were characterized by "smooth" behavior in the open field and elevated plus-maze. LR rats exhibited pronounced locomotor and exploratory activity and high level of anxiety. CCT was followed by inversion of individual behavioral characteristics. LR rats were sluggish and did not display exploratory activity. These changes reflect disintegration of individual components in the general behavioral reaction. HR rats retained such behavioral reactions as sniffing, and motion. Exploratory activity decreased in animals of both groups. However, HR animals demonstrated behavioral and autonomic signs of increased emotional reactivity. The number and duration of grooming reactions and defecation rate increased. These changes reflected psychomotor excitation of animals. In control rats the observed behavioral reactions persisted for 20 days after trauma.

Individual or combined administration of pyrazidol and bemitil increased the survival rate of rats with CCT (Table 1). The number of HR and LR rats receiving pyrazidol and surviving 3 days after CCT was 10% higher compared to untreated animals. Bemitil increased the number of survived HR and LR rats by 44 and 52%, respectively. After combined treatment with pyrazidol and bemitil the survival rate of HR and LR rats surpassed the control by 50 and 66%, respectively.

Behavioral reactions of rats with CCT underwent changes after 20-day therapy (Table 2). Locomotor and exploratory activity of LR animals receiving pyrazidol increased, which was manifested in shortened of the latency of the first movement and increased number of crossed squares, vertical rearing postures, entries into the center of the open field, and explored holes. Pyrazidol had a mild cataleptic effect in HR rats. This drug decreased vertical and horizontal activity of HR animals. After treatment with pyrazidol shortening of the latency of the first movement in HR rats was more pronounced than in LR animals. Behavioral changes in HR rats reflect the sedative effect of pyrazidol.

Bemitil significantly increased locomotor and exploratory activity of HR and LR rats. The observed changes were most pronounced in LR animals. However, these indexes in treated rats did not attained the control level. Combined administration of pyrazidol and bemitil practically did not modulate locomotor and exploratory activity of LR and HR rats.

Emotional reactivity of rats treated in the posttraumatic period underwent considerable changes. Pyrazidol had a sedative effect on HR rats, which was manifested in a decrease in the rate and duration of grooming reactions (Table 2). By contrast, pyrazidol produced a stimulating effect and increased emotional reactivity of LR animals. Bemitil acted as a psycho-

TABLE 1. Animal Survival 3 Days after CCT

Group	CCT (n=30)		CCT and pyrazidol (n=30)		CCT and bemitil (n=19)		CCT and pyrazidol+ bemitil (n=30)	
	abs.	%	abs.	%	abs.	%	abs.	%
HR	14	40	15	50	16	84	27	90
LR	8	27	11	37	15	79	28	93

stimulant in HR and, especially, in LR rats. Combined treatment with pyrazidol and bemitil more significantly improved emotional reactivity of animals, which did not differ from normal.

In animals receiving the test preparations the indexes of anxiety corresponded to the emotional state (Table 2). Pyrazidol increased the time spent in the open arms of the elevated plus-maze, number of entries into the center of the maze, and count of overhanging postures. We observed an increase in the ratio between the count of entries into open arms and total number of transitions. These indexes illustrate the influence of anxiotropic factors and are used for evaluation of anxiety [12]. The observed changes indicate that pyrazidol relieves symptoms of anxiety in animals with CCT. It should be emphasized that these indexes only slightly changed in HR rats. However, changes in the degree of anxiety were significant in LR animals and reflected a potent antidepressant effect of the preparation. Bemetil therapy alleviated symptoms of anxiety in LR and HR rats with CCT. The level of anxiety after monotherapy with bemitil did not reach normal. The psychostimulant action of bemitil was most pronounced in LR rats. Combined administration of bemitil and pyrazidol reduced the degree of anxiety in animals of both groups.

Our results show that individual or combined treatment with the test preparations produces various

TABLE 2. Effect of Individual or Combined Treatment with Pyrazidol and Bemitil on Locomotor Activity, Emotional Reactivity, and Level of Anxiety in Rats with CCT ($M \pm m$, $n=10$)

Index		Intact	CCT	CCT and pyrazidol	CCT and bemitil	CCT and pyrazidol+ bemitil
Locomotor activity						
number of crossed squares	HR	55.2±6.2	38.2±4.2*	25.2±2.1+	48.3±2.6+	58.2±4.7+
	LR	65.2±4.3	21.2±2.2*	26.5±3.3+	59.5±3.4+	64.9±3.3+
latency of the first movement, sec	HR	25±4	30±4*	10±3+	19±5+	26±2+
	LR	15.0±2.2	40±2*	29±2+	12±1+	14±3+
number of entries into the center	HR	10.0±1.3	5.2±2.2*	4.1±1.1+	8.2±2.3+	11.2±4.3+
	LR	7.2±1.2	3.1±1.2*	4.3±2.1+	5.6±1.9+	8.4±2.6+
number of rearing postures	HR	20.0±2.2	14.2±3.4*	12.1±1.4+	15.2±2.7+	19.2±5.1+
	LR	42.2±3.2	2.2±1.2*	3.5±2.3+	35.4±2.3+	45.2±2.7+
number of explored holes	HR	5.0±0.9	1.0±0.3*	0.6±1.3+	4.1±1.3+	5.5±1.3+
	LR	8.2±4.2	0.5±0.2*	1.2±1.4+	7.4±2.3+	7.9±4.2+
Emotional reactivity						
number of grooming reactions	HR	2.0±0.1	3.2±0.3*	0.8±0.2	1.6±0.3+	1.8±0.2+
	LR	3.5±0.2	0.9±0.2*	1.4±0.4+	2.0±0.2+	2.6±0.3+
duration of grooming reactions, sec	HR	25±4	29±2*	8±3	18±4+	20±2+
	LR	32±2	5±2*	8±4+	19±2+	28±4+
defecation rate	HR	2.0±0.3	3.1±0.3*	1.3±0.4	1.6±0.2+	1.8±0.2+
	LR	2.8±0.2	0.8±0.2*	1.2±0.2+	2.2±0.2+	2.6±0.4+
Anxiety						
time spent in the open arm, sec	HR	53±3	22±5*	29±4	45±6+	56±4
	LR	78±4	17±5*	42±6+	48±3+	73±3+
time spent in the closed arm, sec	HR	222±6	278±5*	271±3	255±3+	244±4+
	LR	247±7	283±4*	258±2+	252±4+	227±5+
looking out from the closed arm	HR	7.4±0.3	2.2±0.4*	2.7±0.4	4.8±0.3+	6.9±0.4+
	LR	5.2±0.2	1.2±0.2*	2.6±0.6+	4.5±0.2+	5.6±0.2+
number of entries into the center	HR	4.4±0.2	1.2±0.3*	1.4±0.3	2.2±0.4+	4.6±0.4+
	LR	2.3±0.3	0.4±0.2*	0.8±0.1+	1.6±0.2+	2.5±0.1+
number of overhanging postures	HR	6.2±0.2	1.4±0.3*	1.7±0.2	3.4±0.3+	5.9±0.2+
	LR	4.6±0.3	0.9±0.5*	2.4±0.4+	3.2±0.2+	4.4±0.3+

Note. *$p<0.05$ compared to intact animals; +$p<0.05$ compared to CCT.

TABLE 3. Effect of Pyrazidol, Bemitil, and Their Combination on Parameters of Energy Metabolism, LPO, and Antioxidant Systems in the Brain of Rate after CCT ($M \pm m$, $n=10$)

Index		Intact	CCT*	CCT and pyrazidol	CCT and bemitil+	CCT and pyrazidol+ bemitil+
Conjugated dienes, μmol/g	HR	21.58±0.26	45.12±0.32	37.25±0.21	27.89±0.16	20.56±0.21
	LR	25.02±0.22	56.14±0.82	41.35±0.22+	29.35±0.13	23.22±0.23
MDA, μmol/g	HR	6.18±0.15	15.14±0.12	10.27±0.12+	7.72±0.11	7.01±0.17
	LR	7.34±0.14	20.82±0.14	12.12±0.11+	8.13±0.12	8.14±0.14
Reduced glutathione, μmol/g	HR	42.12±0.43	22.62±0.40	29.79±0.21	37.15±0.21	39.85±0.27
	LR	30.52±0.22	15.23±0.62	21.02±0.23+	24.12±0.14	28.87±0.24
SOD, U/mg protein	HR	3.21±0.07	1.25±0.06	1.57±0.04	2.04±0.07	2.89±0.06
	LR	2.02±0.06	0.88±0.03	1.22±0.03+	1.97±0.04	2.18±0.04
Creatin phosphate, μmol/g	HR	4.25±0.04	1.12±0.04	2.28±0.12	3.18±0.04	3.69±0.04
	LR	3.24±0.05	0.87±0.05	1.98±0.03+	2.87±0.05	2.98±0.03
ATP, μmol/g	HR	3.68±0.14	2.12±0.9	2.45±0.12	3.18±0.12	3.78±0.05
	LR	2.43±0.15	1.29±0.05	1.81±0.11+	2.11±0.07	2.12±0.02
Lactate, μmol/g	HR	2.11±0.04	6.24±0.03	5.12±0.12+	2.88±0.22	3.12±0.05
	LR	3.89±0.11	8.43±0.06	6.52±0.09+	4.54±0.05	4.12±0.06
Pyruvate, μmol/g	HR	0.38±0.01	0.14±0.02	0.19±0.01+	0.25±0.01	0.32±0.01
	LR	0.25±0.02	0.09±0.01	0.14±0.01+	0.19±0.01	0.22±0.01

Note. *$p<0.05$ compared to intact animals; +$p<0.05$ compared to CCT.

effects on behavioral reactions of rats with CCT. Pyrazidol had a typical "balanced" effect, increased locomotor activity, and reduced anxiety and emotional reactivity in LR rats. However, the preparation produced sedative and mild cataleptic effects in HR animals. As distinct from pyrazidol, bemitil did not decrease locomotor and emotional activity in HR rats. Moreover, bemitil had a psychostimulant action and increased locomotor and emotional activity in LR animals. During combined treatment, pyrazidol and bemitil had an additive effect on behavioral reactions of animals phenotypically differing by the resistance to hypoxia. These rats did not differ from intact animals in locomotor and orientation-and-exploratory activity, emotional reactivity, and level of anxiety.

Behavioral reactions serve as a general criterion for functional and metabolic changes. It can be hypothesized that bemitil possessing metabolic activity produces a strong therapeutic effect during treatment of CTT consequences. Bemitil possesses energy-stimulating and antioxidant properties. The protective effect of this preparation during CCT requires further investigations [3,5]. We studied the metabolic effect of individual or combined treatment with pyrazidol and bemitil in rats with CCT. The test preparations produced various cerebroprotective effects (Table 3). Pyrazidol was more potent in preventing the development of metabolic disturbances in the brain of LR rats. This preparation decreased the contents of conjugated dienes and MDA by 26 and 42%, respectively. Moreover, pyrazidol increased the content of reduced glutathione and SOD activity by 38 and 39%, respectively. Stabilization of the energy potential in the brain of LR rats receiving pyrazidol manifested in increased contents of creatine phosphate and ATP (by 128 and 40%, respectively). The preparation prevented accumulation of excess lactate, but increased pyruvate content by 56%. The metabolic state in the brain of HR rats receiving pyrazidol tended to normal. It should be emphasized that bemitil was more potent than pyrazidol during the therapy of animals with CCT. In animals of both groups bemitil prevented accumulation of LPO products in the brain, suppression of the antioxidant system, and development of metabolic acidosis and energy deficit. The therapeutic effect of bemitil was more pronounced in LR rats. However, metabolic indexes in bemitil-treated rats did not reach the normal. After combined administration of pyrazidol and bemitil, the metabolic state of the brain in treated rats did not differ from that in intact animals. Combined treatment with the test preparations most significantly normalized the content of LPO products, SOD activity, and amount of macroergic compounds in LR rats.

These data indicate that the phenotypic resistance to hypoxia plays a role in the formation of behavioral reactions and metabolic changes in the brain after

CCT. Therefore, individuals differing in the resistance to hypoxia should receive individual pharmacological correction. Pyrazidol monotherapy for CCT consequences produced a "balanced" effect in HR rats. This preparation had an anxiolytic effect and reduced psychomotor excitation of HR rats in the posttraumatic period. However, pyrazidol produced an activating effect in LR animals.

Bemitil had the same effects in HR and LR rats with CCT. The preparation normalized individual behavioral characteristics and metabolism in the brain. However, bemitil therapy was more preferable to LR animals. The mild psychostimulant effect of bemitil in LR rats was more pronounced than in HR animals.

During combined treatment, pyrazidol and bemitil produced an additive corrective effect in HR and LR rats with CCT consequences. This treatment normalized behavioral reactions and prevented the development of metabolic disturbances in the brain. Taking into account the additive effect of drugs, it cannot be excluded that the antidepressant pyrazidol can be used in a lower dose during combined treatment with bemitil. Sometimes, it is impossible to evaluate phenotypic characteristics of the organism by the resistance to hypoxia. Then, the correction of disturbances in higher nervous activity and metabolic changes in the brain during CCT should involve combined treatment with the antidepressant pyrazidol and antihypoxant bemitil.

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