Neuroprotective Properties of Afobazole in Repeated Hemorrhagic Stroke Modeling in Aged Rats

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Intraperitoneal administration of afobazole in a dose of 0.1 mg/kg over 2 weeks after repeated modeling of intracerebral post-traumatic hematoma reduces animal mortality, decreases motor coordination disturbances, and improves learning and memory processes in rats.

Key Words: *afobazole; intracerebral posttraumatic hematoma; neuroprotection; intracerebral posttraumatic hematoma; neurological deficit; memory*

Afobazole (5-ethoxy-2-[2-(morpholino)-ethylthio] benzimidazole dihydrochloride) was synthesized and pharmacologically investigated in V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences; in 2005 it was registered in the Russian Federation as a selective anxiolytic drug [5].

Experiments showed that selective anxiolytic properties of afobazole are determined by prevention of stress-induced decline of benzodiazepine reception, which results in normalization of GABA transmission [7]. At the same time, it was shown that the compound possesses antioxidant activity, positively modulates NO production [13], and restores BDNF level after its stress-induced reduction [9].

We studied neuroprotective properties of afobazole under conditions of local ischemic damage produced by ligation of the medial cerebral artery. It was demonstrated that the drug limits the area of ischemic damage and promotes normalization of pathomorphological changes of the brain tissue observed under conditions of local ischemia [13].

Then, the protective properties of afobazole were revealed in culture of hippocampal neurons in modeled oxidative stress and glutamate toxicity [3]. Subsequent *in vivo* experiments established neuroprotective action of afobazole during simulation of hemorrhagic stroke (HS) [1] and focal brain ischemia [10]. Afobazole also enhanced blood supply to ischemic brain [11].

Analysis of receptor interactions of afobazole showed that the compound is a ligand of σ_1-receptor (IC50-7.1E-06M), which can explain its cytoprotective properties [6,14].

Our previous studies with HS simulation showed that intraperitoneal administration of afobazole in doses of 1.0 and 0.1 mg/kg 6 h after surgery and then daily twice a day for 2 weeks significantly reduced manifestation of post-stroke disorders. In a dose of 1 mg/kg, the drug completely prevented animal death, improved the indices of neurological deficit, and enhanced cognitive functions [8].

Thus, the data obtained in *in vivo* and *in vitro* experiments provide strong evidence on neuroprotective action of afobazole. Since repeated stroke is one of frequent complications, particularly in elderly patients [12], the aim of the study was to evaluate the neuroprotective properties of afobazole in the model of repeated HS in aged rats.

MATERIALS AND METHODS

Experiments were conducted on 15-month-old outbred albino male rats weighing 400-450 g. The animals survived after primary HS were kept under standard vivarium conditions with free access to food and water at 12-h illumination regimen for 12 months before the start of the experiment.

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TABLE 1. Effect of Afobazole (0.1 mg/kg) on Animal Survival after HS (*M*±*m*)

Group of animals	Total animal death		Number of dead animals over 2 weeks after HS (dynamics)							
			day 1		day 3		day 7		day 14	
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Group 1 (*n*=12)	0	0	0	0	0	0	0	0	0	0
Group 21 (*n*=8)	5	62.5*	3	37.5	2	40	0	0	0	0
Group 3 (*n*=9)	3	33.3+	0	0	3	33.3	0	0	0	0

Note. $p \leq 0.05$ compared to: *group 1, +group 2 (Fisher's exact test).

Repeated local brain hemorrhage was reproduced according to the method of intracerebral posttraumatic hematoma [4].

Brain tissue of anesthetized rats was destructed after craniotomy using a specific device (mandrin-knife) and stereotaxis in the region of the inner capsule and blood (0.02-0.03 ml) taken sublingually was injected into the damaged area 2-3 min later. In such a way, local autohemorrhagic stroke was modeled in the region of the inner capsule (diameter 2 mm, depth 3 mm) without sufficient damage to upper brain structures and neocortex.

The number of survived animals, motor coordination, muscular tone, and exploratory behavior were recorded 24 h after surgery.

Then rats were divided into 3 groups: group 1 comprised sham-operated rats (SO) subjected to craniotomy under anesthesia; group 2 consisted of animals with HS; and group 3 included animals with HS receiving afobazole in a dose of 0.1 mg/kg. Injections were given intramuscularly 6 h after surgery, and subsequently twice a day every day for 2 weeks. Control animals (groups 1 and 2) received physiological saline in an equivalent volume.

The dynamics of disturbances induced by intracerebral post-traumatic hematoma and the effects of afobazole were evaluated for 14 days; animal survival, behavior, and general state were assessed on days 1, 3, 7 and 14 after surgery, using a battery of methods described previously [2].

Statistical data analysis was performed by Student's *t* test and Fisher's exact test using Biostat software.

RESULTS

No animal death was observed in group 1 over the last 2 weeks after repeated stroke.

In group 2, about 37% animals died during the first day and 62.5% were dead by day 14. Injection of afobazole in a dose of 0.1 mg/kg according to the specified scheme reduced the total death to 33% (Table 1).

In group 1, disorders of motor coordination were observed, which completely disappeared by day 7. In group 2, motor coordination disturbances were noted in 80 and 67% survivors on days 1 and 14 after surgery, respectively.

Afobazole in the dose of 0.1 mg/kg attenuated disturbances of motor coordination in rats on day 1 after stroke and completely eliminated them by day 7 (Table 2).

Weakening of the muscular tone within days 1-14 was noted in 80-100% animals of group 2, whereas in

TABLE 2. Effect of Afobazole (0.1 mg/kg) on Motor Coordination in Rotarod Test after HS (*M*±*m*)

Group of animals	Number of animals failing to hold on the rotating rod (3 rpm) for 2 min							
	day 1		day 3		day 7		day 14	
	abs.	%	abs.	%	abs.	%	abs.	%
Group 1 (*n*=12)	5	41.7	2	16.7	0	0	0	0
Group 2 (*n*=5)	4/5	80	2/3	66.7	2/3	66.7	2/3	66.7
Group 3 (*n*=9)	3/9	33.3	3/6	50	0/6	0	0/6	0

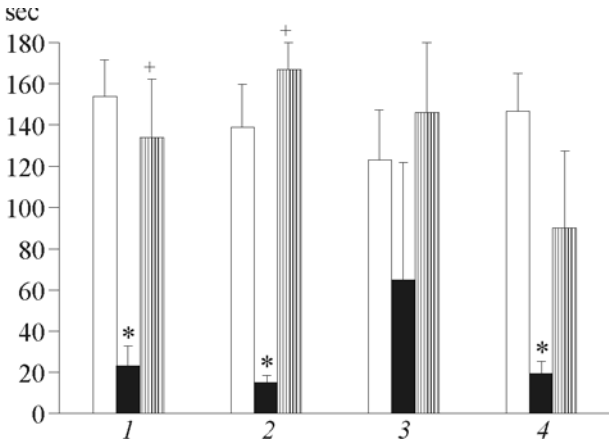

Fig. 1. Effect of afobazole on PAR retrieval in rats with intracerebral posttraumatic hematoma. Light bars: group 1; dark bars: group 2; shaded bars: group 3. *1*) 24 h after training; *2*) on day 3 after surgery; *3*) on day 7 after surgery; *4*) on day 14 after surgery. Ordinate: latency of entry into dark chamber. Here and on Fig. 3: $p<0.05$ compared to: *group 1, +group 2 (*t* test).

group 3 treated with 0.1 mg/kg afobazole weakening of the muscular tone on day 1 was observed in 42-50% animals and by day 14 this parameter decreased to 17% (Table 3).

It was found that in SO animals the hole reflex was not impaired. Rats with HS also demonstrated unimpaired hole reflex, but its latency was slightly increased. Afobazole in a dose of 0.1 mg/kg reduced the latency of the hole reflex, *i.e*. improved conditioning (16.1±4.3 *vs*. 33.8±10.4 sec in group 2 rats).

Evaluation of the effect of HS on memory showed that 1 day after training 83% group 1 rats avoided entrances into the dark chamber where they received electric footshock during training, while in group 2, 100% animals entered the dark chamber. Experiments with passive avoidance response (PAR) retrieval on day 14 after surgery showed that memory trace was not retained in group 2 rats, while in group 1 this parameter was 75%.

Afobazole in a dose of 0.1 mg/kg significantly increased the latency of entry into the dark chamber in the PAR paradigm in group 3 rats on days 1 and 3; on days 7 and 14, the increase in PAR latency was longer than group 2 (Fig. 1).

Analysis of the orientation and exploratory behavior in the open field test revealed an almost 4-fold decrease in motor activity and exploratory behavior after surgery in group 2 rats compared to group 1 animals, which persisted throughout the observation period.

Afobazole in the dose of 0.1 mg/kg significantly increased the total behavioral parameters on days 1-14 after HS to a level observed in group 1 (Fig. 2).

Estimation of animal behavior after HS in the elevated plus-maze on days 1 and 7 revealed the absence of transitions into open arms. This index slightly increased by day 14 after HS.

Afobazole in a dose of 0.1 mg/kg increased the number of transitions into open arms on day 1 after HS up to the level observed in group 1; on days 7 and 14, the time spent in open arms of the maze and the number of entries into open arms increased (Fig. 3).

Thus, afobazole, administered intraperitoneally in a dose of 0.1 mg/kg 6 h after surgery and then twice

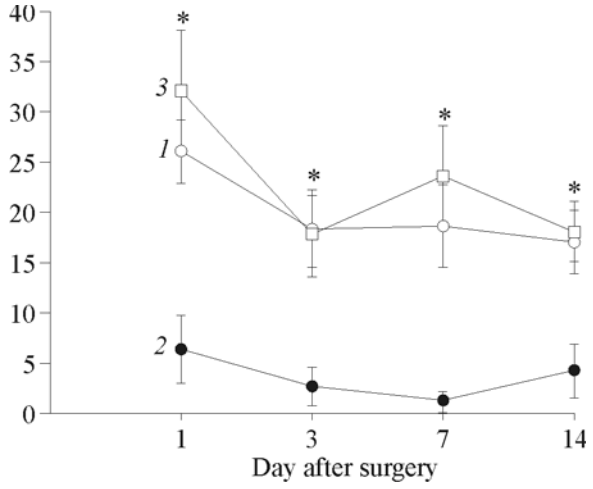

Fig. 2. Effect of afobazole on total motor activity in the open field test. *1*) group 1; *2*) group 2; *3*) group 3. *$p<0.05$ compared to group 2 (*t* test).

TABLE 3. Effect of Afobazole (0.1 mg/kg) on Muscular Tone of Animals in Horizontal Bar Test after HS (*M*±*m*)

Group of animals	Number of animals failing to pull up on the horizontal bar							
	day 1		day 3		day 7		day 14	
	abs.	%	abs.	%	abs.	%	abs.	%
Group 1 (*n*=12)	5	41.7	3	25	0	0	0	0
Group 2 (*n* =5)	4/5	80	3/3	100	3/3	100	3/3	100
Group 3 (*n* =9)	4/9	42	3/6	50	1/6	16.7	1/6	16.7

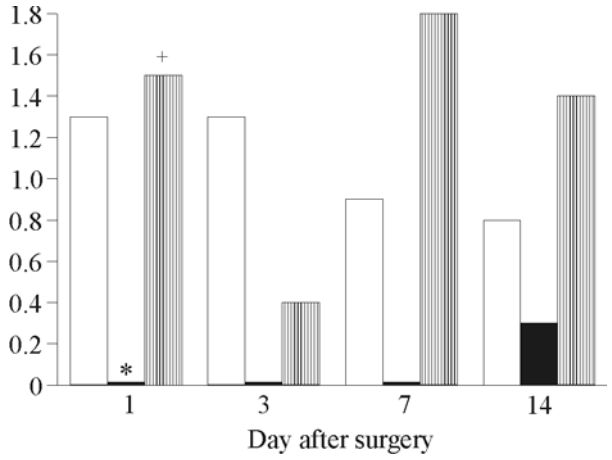

Fig. 3. Effect of afobazole on animal behavior in elevated plus maze test. Light bars: group 1, dark bars: group 2, shaded bars: group 3. Ordinate: number of transitions into open arms.

a day every day for 2 weeks prevented animal death, improved impaired memory and PAR retrieval, and restored motor coordination. Complex influence of the drug on negative consequences of the modeled disease is thus proved.

The data obtained in this study agree with previous investigations. So, comparison of the pharmacological effects of afobazole on the models of single [8] and repeated stroke showed that the preparation improved animal survival by 42 and 30%, respectively. On day 14 after first stroke, the drug increased muscular tone and improved motor coordination by 40 and 17% [8] and after repeated stroke by 80 and 60%, respectively. Both experiments showed improvement of impaired memory and PAR retrieval.

Our findings agree with the data on the primary mechanism of afobazole action, interaction with σ_1-receptors [6]. Agonistic influence on σ_1-receptors promotes recovery of the phospholipid microenvironment of proteins in the outer neuronal membranes, regulates Ca^{2+} balance in the cell, prevents glutamate excitotoxicity, stimulates production of nerve growth factor, and decreases excessive NO generation [6]. Our data agree with experimental data [15] demonstrating neuroprotective properties of other σ_1-receptor agonists.

Thus, the results of our study and published data suggest that afobazole is a promising pharmacological agent for pharmacotherapy of stroke.

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