Neuroprotective Effects of Afobazol in Experimental Cerebral Hemorrhage

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The study of novel selective anxiolytic afobazol on rats with experimental intracerebral post-traumatic hematoma (cerebral hemorrhage) demonstrated its efficiency in a dose of 5 mg/kg applied by a single or repeated administration for 2 weeks. The preparation significantly decreased the incidence of neurological disturbances in most rats (pareses, paralyses, convulsive movements, lateral posture). The therapeutic course of afobazol improved survival rate. Afobazol improved learning and memory in rats with cerebral hemorrhage in the conditioned passive avoidance test and positively affected motor activity in the open field test, which was documented by significant increase in total motor activity indices. The effects of afobazol were more pronounced after course treatment.

Key Words: *afobazol; cerebral hemorrhage; neural deficiency; learning; memory*

Disturbances of cerebral circulation, ischemic strokes and cerebral hemorrhages (CH) belong to leading factors of mortality. The consequences of stroke lead to disablement due to neurological deficiency and disturbances of mnestic and mental functions [3,10]. The most dangerous and resistant to treatment cerebral lesions in CH are caused by blood penetration into adjacent cerebral tissues during intracerebral hemorrhages, hemorrhages into the cerebral ventricles, subarachnoid space, extradural and subdural areas because of pronounced anatomic and physiological shifts, metabolic disturbances (including energy and substrate deficiency) ionic imbalance, glutamate excitotoxicity, oxidative stress, disturbances of enzyme functions, anoxic depolarization of the membranes and the death of neurons [3,8,9].

Complex mechanisms of stroke pathogenesis necessitate the development of adequate pharmacotherapeutic methods aimed at restoration of cell homeostasis.

To this end, a novel selective anxiolytic afobazol was synthesized at V. V. Zakusov State Research Institute of Pharmacology [6].

The mechanism of its action is based on restoration of disturbed functions of $GABA_A$-benzodiazepine receptor complex [6].

Afobazol possesses antiradical potency, prevents ischemia-induced intensification of NO production in the brain [7], and exhibits protective properties in simulated glutamate toxicity *in vitro*. These data corroborate indications on the common links in the mechanisms of anxiogenesis and stroke pathogenesis, and explain importance to study the neuroprotective properties of afobazol.

In this paper, we examined the effects of afobazol on survival rate and CNS function in rats with intracerebral post-traumatic hematoma.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino male rats weighing 200-250 g. The rats were

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maintained in a vivarium of V. V. Zakusov State Research Institute of Pharmacology under 12:12 h light-dark cycle and were given food briquettes and water *ad libitum*. The experiments were performed during fall-winter period.

Local CH was modeled as described previously [1,2,4]. The rats were intraperitoneally narcotized with chloral hydrate (400 mg/kg). Special mandrin knife and stereotactic technique were used to destruct cerebral tissue in internal capsule. In 2-3 min a portion of blood (0.02-0.03 ml) drawn from sublingual region was injected into this destructed area.

The rats were subdivided into 4 groups. Group 1 and group 2 comprised sham-operated (SO) and CH rats, respectively. Group 3 CH rats received single intraperitoneal injection of 5 mg/kg afobazol 3.5-4.0 h after surgery. Group 4 CH rats received 5 mg/kg afobazol intraperitoneally two times a day with 4 h interval during the first 3 days after surgery and single daily injection in the same dose for the following 11 days.

Functional state of the nervous system was assessed as described earlier [1]. Neurological deficiency was rated using a modified McGrow scale, taking into account minor disturbances (inertia, retarded movements, general weakness, ptosis, and semiprosis) and severe abnormalities (paralyses and pareses of extremities, lateral posture). The locomotor, orientation, and exploratory activities were examined in the open field test. Learning and memory were assessed by conditioned passive avoidance response (CPAR) [1].

The data were analyzed statistically using Biostatistica software. The intergroup data were compared using Mann—Whitney *U* test, Fisher angular conversion, Student's *t* test, and the χ^2 test. The differences were significant at $p \leq 0.05$.

RESULTS

In the group of SO rats, 100% survived to day 7 after surgery, but 15% died during the following week. In group 2 rats (CH without treatment), 35% rats died during the first 3 days, and 50% died to the end of the experiment. Single injection of afobazol had no effect on survival rate (Fig. 1, *a*). The course of afobazol significantly decreased mortality rate to 3, 7, and 14 days after the surgery (Fig. 1, *a*).

Analysis of neurological deficiency by McGrow scale revealed neurological abnormalities in all operated rats. Severe symptoms like convulsive movements, paralyses of extremities, and lateral posture were observed in 10% SO rats. In the group of untreated CH rats, severe symptoms were documented in 30, 65, and 70% rats on postsurgery days 1, 7, and 14, respectively. Single injection of afobazol produced a protective effect, which was more pronounced after course treatment: in group 4 rats, severe neurological deficit on postsurgery day 14 was documented in only 8% (Fig. 1, *b*).

On postsurgery day 1, decreased motor activity was observed in the group of untreated CH rats in comparison with SO rats. On day 14, the parameter of motor activity in the group of untreated CH rats remained at the low level (Table 1). Both single and course administration of afobazol prevented the drop of motor activity in CH rats in open field test (Table 1).

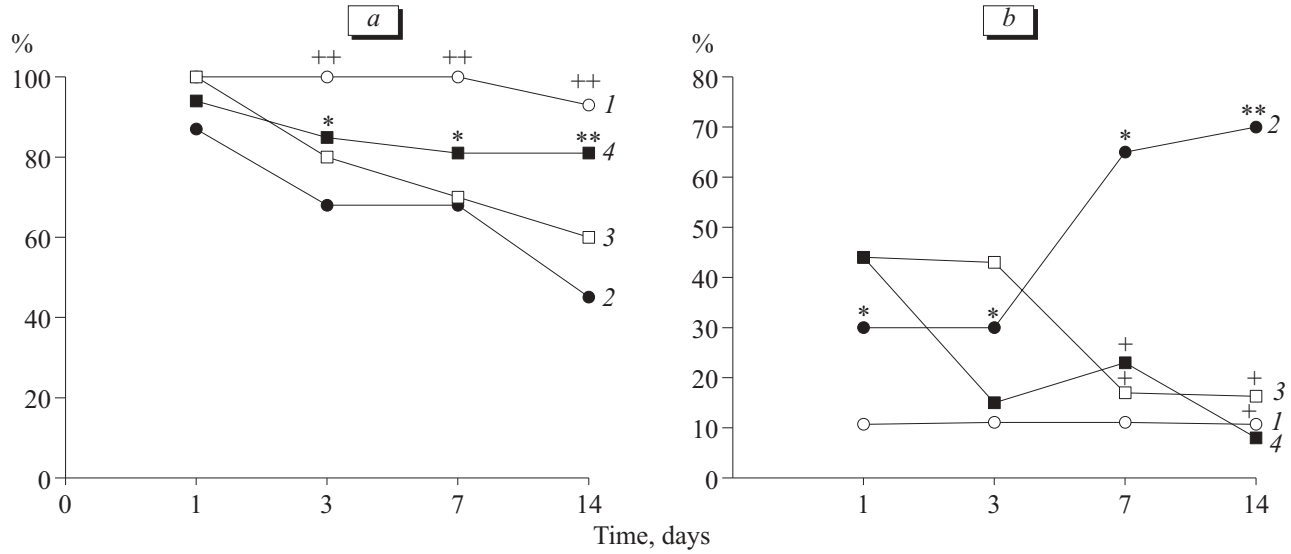

Fig. 1. Effect of afobazol on survival rate (*a*) and development of severe neural disorders (*b*) in rats after cerebral hemorrhage (CH). *1*) sham-operated (SO) rats; *2*) CH rats; *3*) single injection of afobazol; *4*) therapeutic course of afobazol. **p*<0.05, ***p*<0.01 compared to SO rats (χ^2 test); ^{+}p<0.05, ^{++}p<0.01 compared to CH rats (Fisher angular coefficient).

TABLE 1. Effect of Afobazol on Motor Activity of CH Rats in Open Field Test (*M*±*m*)

Group	Horizontal motor activity	Vertical motor activity	Hole exploration	Total
Postsurgery day 1				
SO	18.1±2.6	4.6±0.9	2.1±0.5	24.8±3.3
CH	9.0±3.6*	1.7±0.9*	0.1±0.1	10.7±2.4*
afobazol (single injection)	32.7±7.5^{++}	5.3±1.5	1.7±0.7	39.3±9.0^{+}
afobazol (course)	30.2±4.5^{+}	3.9±1.2	1.4±0.3	35.4±2.6^{++}
Postsurgery day 14				
SO	8.8±1.7	2.4±0.7	2.6±0.4	13.1±2.3
CH	7.2±1.5	2.9±1.0	1.8±0.6	11.9±2.4
afobazol (single injection)	15.8±6.2	3.1±1.3	0.4±0.1	19.2±5.3
afobazol (course)	19.8±3.3*	4.8±1.2	0.1±0.1	24.7±4.2*

Note. *$p \le 0.05$ compared to SO rats; $^{+}p \le 0.05$, $^{++}p \le 0.01$ compared to CH rats (Student's *t* test).

TABLE 2. Effect of Afobazol on Reproduction of CPAR in CH Rats 24 h and 14 Days after Training (*M*±*m*)

Group	Reproduction of CPAR			
	24 h		14 days	
	latency of dark chamber entry, sec	number of rats that did not enter the dark chamber, %	latency of dark chamber entry, sec	number of rats that did not enter the dark chamber, %
SO	169.1±7.3	100	79.9±6.8	50
CH	137.5±17.6	76**	35.7±5.4*	11^{o}
Afobazol (single injection)	>180	100^{+}	83.0±12.2^{+}	40^{+}
Afobazol (course)	140.4±17.4	83	96.3±10.3^{++}	50**

Note. *$p \le 0.05$ (Student's *t* test) and **$p \le 0.05$ (Mann—Whitney *U* test) compared to SO rats; $^{+}p \le 0.05$ (Student's *t* test) and $^{++}p \le 0.01$ (Mann—Whitney *U* test) compared to CH rats.

On day 14, the memory of CH rats was impaired compared to SO rats (Table 2). Either single or course administration of afobazol prevented this memory deficit, the effect was more pronounced after course treatment (Table 2).

Thus, afobazol produced neuroprotective effects, which were more pronounced after course treatment. These data substantiate further study of afobazol as a part of stroke pharmacotherapy, because this agent combines the anxiolytic and neuroprotective properties.

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