Study of the Effects of Preparation Containing Ultralow Doses of Antibodies to S-100 Protein in Experimental Hemorrhagic Stroke

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, Suppl. 1, pp. 170-173, September, 2009
Original article submitted August 1, 2008

Ultralow doses of antibodies to S-100 protein increased rat survival, reduced neurological deficit, eliminated myorelaxation, and improved movement coordination and cognitive functions in rats with experimental hemorrhagic stroke; the efficiency of the preparation was not inferior to that of nimodipine. In contrast to nimodipine, ultralow doses of antibodies to S-100 protein exhibited pronounced anxiolytic properties.

Key Words: *hemorrhagic stroke; nimodipine; antibodies to S-100 protein; ultralow doses*

Hemorrhagic stroke (HS) is the most grave and disabling type of stroke constituting 10-15% of the total number of strokes. Mortality in HS is 25-80%; 35-50% patients die within 30 days after cerebral hemorrhage (half of them within the first 2 days) [3]. The therapy of HS includes a complex of measures aimed, first of all, at elimination of hematoma and prophylactics of brain edema during the first few hours after stroke. At later terms, the therapy is aimed at recovery of disturbed brain functions (with neuroprotective drugs and nootropics) and prevention of secondary stroke (with angioprotectors, Ca^{2+}-channel blockers, antiaggregants, *etc*.) [7]. It was previously demonstrated that ultralow doses of antibodies to S-100 protein (ULD of anti-S100) exhibit neuroprotective activity under conditions of ischemic stroke [4].

Here we studied the effects of ULD of anti-S100 under conditions of experimental HS.

MATERIALS AND METHODS

HS was modeled on outbred albino male rats weighing 200-250 g [2]. In rats narcotized with Nembutal (40 mg/kg intramuscularly), soft tissues and the periosteum in the central parietal area of the skull were removed. A hole with a diameter of 1 mm was drilled on the left side of the skull 1.5-1.8 mm caudally from bregma and 2.5-3.0 mm laterally from the sagittal suture. A mandarin was inserted into this hole to a depth of 4 mm, the brain tissue near the internal capsule was destructed and after 2-3 min the blood drawn from sublingual vessels (0.02-0.03 ml) was injected into the site of destruction. Morphological study showed that these manipulation led to the development of local bilateral stroke in the area of the internal capsule (diameter 2 mm, depth 3 mm) without considerable damage to the surface structures (*e.g.* neocortex).

ULD of anti-S100 (2.5 ml/kg), Ca^{2+}-channel blocker nimodipine (0.1 mg/kg), or distilled water (2.5 ml/kg) were administered intragastrically for 14 days. The first injection was made 4 h after HS modeling. Controls underwent sham-operation: scalping of the central parietal area and drilling the hole without damage to brain structures.

Rat survival was evaluated over 14 days; neurological and emotional status, muscular tone, coordination of movement, and cognitive functions were evaluated on days 1, 3, 7, and 14.

Neurological deficiency was evaluated by Mc Grow scale with some modifications [1]. The number of rats

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0007-4888/09/1483-0530

with mild (score 0.5-2.5) and severe (score 3-10) neurological symptoms was determined.

Coordination of movements was studied using the rotarod test [6,9]. Inability of maintaining the balance on a rod with a diameter of 4 cm rotating at a rate of 3 rpm over 2 min was considered as disturbed coordination of movements. Muscular tone disturbances were evaluated using the pull-up test on a horizontal bar elevated at a height of 20-30 cm above the floor [5].

Cognitive functions were evaluated by conditioned passive avoidance reaction (PAR) [5,8]. The rat was placed on a brightly illuminated platform (25×7 cm) with its tail to a square hole (6×6 cm) leading to a chamber with electrode floor (40×40×40 cm). Due to burrow reflex, the rat after finding the entry into the dark chamber moved into it and spent most time there. The latency of the first entry into the dark chamber and the total time spent there were recorded over 180 sec after placing the animal into the experimental setup. Then, the door was closed and the rat restrained in the dark chamber was subjected to inescapable electrical painful stimulation (ten 1-sec pulses, 0.45 mA current, 2 sec interval between pulses).

Rat anxiety was evaluated in an elevated plus maze (EPM) [5,6,11] over 5 min by the number of entries into open arms and the time spent there.

The significance of differences between the groups was evaluated using Student *t* test.

RESULTS

Experimental HS led to death of 50% animals by day 14 after surgery. ULD of anti-S100 significantly increased rat survival (by 20%, $p<0.05$); the effect of nimodipine on rat survival was insignificant.

HS induced the development of neurological disturbances in 100% rats, myorelaxation in 50%, and disturbances in coordination of movements in 30% animals (Table 1). The severity of neurological deficit gradually decreased over 14 days, mild (flaccid movements, weakness in limbs) and severe (riding movements, pareses of the limbs, palsy) disturbances persisted in 60% and 20% rats, respectively. Both nimodipine and ULD of anti-S100 reduced neurological symptoms of HS at all terms of the study (Table 1). On day 14 after HS, nimodipine and ULD of anti-S100 decreased the number of animals with mild disturbances by 1.4 and 1.8 times ($p<0.05$), respectively; nimodipine completely eliminated severe disturbances, while ULD of anti-S100 1.5-fold reduced their incidence. ULD of anti-S100, in contrast to nimodipine, decreased also the number of rats with disturbed coordination of movements and myorelaxation by 1.7 and 2 times, respectively ($p<0.05$; Table 1).

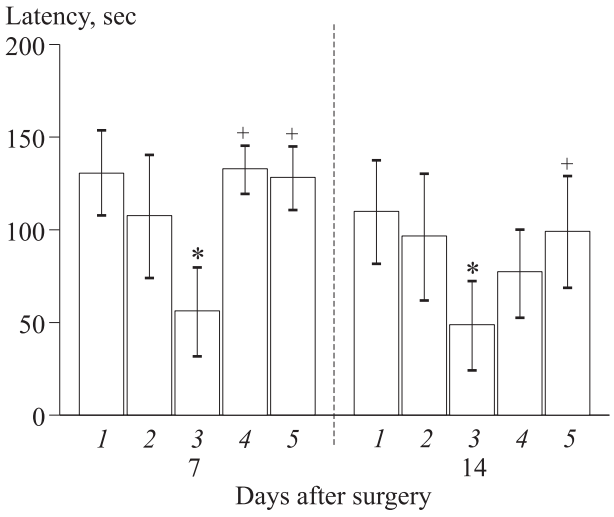

Fig. 1. Effect of nimodipine and ULD of anti-S100 on cognitive functions of rats after HS in PAR test. Here and on Fig. 2: *1*) intact; *2*) control; *3*) HS+distilled water; *4*) HS+nimodipine; *5*) HS+ULD of anti-S100. $p<0.05$ compared to: *control, +HS+distilled water.

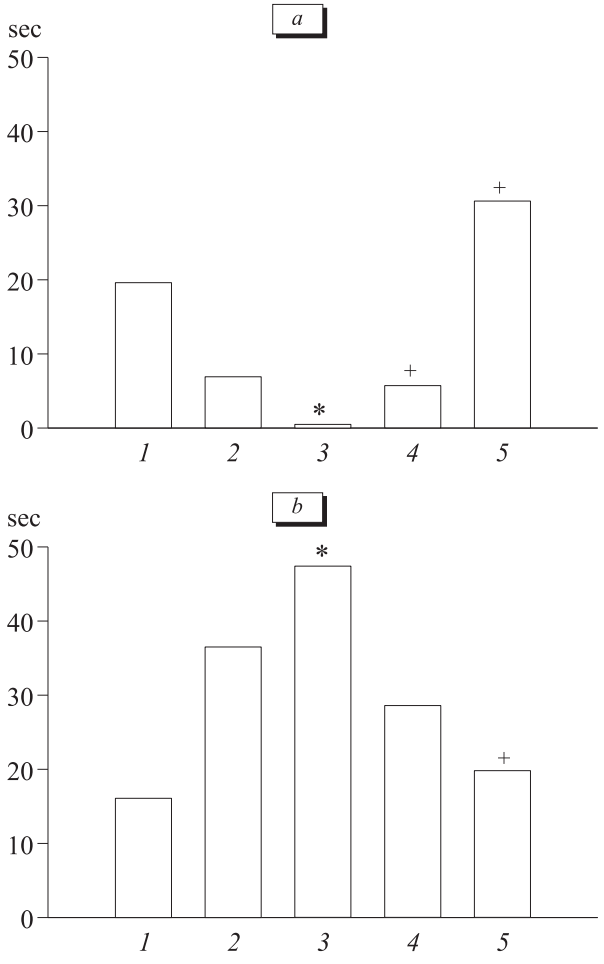

Fig. 2. Effect of nimodipine and ULD of anti-S100 on EPM behavior of rats after HS. *a*) time spent in open arms; *b*) time spent on the central platform.

TABLE 1. Effect of Nimodipine and ULD of Anti-S100 on Neurological Status, Myorelaxation, and Coordination of Movements in Rats after HS (%)

Parameter	Group	Days after surgery			
		1	3	7	14
Severe neurological disturbances	Control	0	0	0	0
	HS+distilled water	44*	12	0	20
	HS+nimodipine	20	24	14	0
	HS+ULD of anti-S100	30+	33+	14	14
Mild neurological disturbances	Control	60	40	20	20
	HS+distilled water	100*	100*	86*	60*
	HS+nimodipine	50+	50	43+	43+
	HS+ULD of anti-S100	60	66	50+	43+
Animals unable to pull-up on the bar	Control	20	10	10	10
	HS+distilled water	30	50*	43*	50*
	HS+nimodipine	20	50*	71*	50*
	HS+ULD of anti-S100	10	10+	24	25
Animals unable to stay on rotating rod	Control	10	0	10	10
	HS+distilled water	30	25	29	20
	HS+nimodipine	20	25	43*	17
	HS+ULD of anti-S100	9+	18	12	12

Note: $p<0.05$ compared to: *control, +HS+distilled water.

On days 7 and 14 after HS, PAR performance was appreciably impaired (by 2 times, $p<0.05$), which attested to disturbances in cognitive functions. Both nimodipine and ULD of anti-S100 completely restored PAR performance on day 7, but on day 14 this effect persisted only in rats receiving ULD of anti-S100 (Fig. 1).

Apart from neuroprotective effect, ULD of anti-S100 exhibited anxiolytic activity: in the EPM test the preparation not only eliminated symptoms of anxiety resulting from HS, but also significantly increased the time spent in open arms compared to that in intact animals (by 1.6 times, $p<0.05$; Fig. 2).

Thus, ULD of anti-S100 increased rat survival and eliminated neurological disturbances developing in rats after HS. Taking into account the positive results obtained previously in studies of the antiischemic effect of the preparation on the model of ischemic stroke [6], the preparation can be recommended as a potential drug for the treatment of both ischemic stroke and HS.

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