ANTI-SCHEMIC PROTECTION OF THE BRAIN BY ACELIZINE, A WATER-SOLUBLE ASPIRIN

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UDC 616.831-005.4-084:[615.212.3:547.587.11]:615.456.1]-092.9

KEY WORDS: acelizine; brain; ischemia.

The problem of protection of the brain against ischemic damage remains an extremely urgent one in modern medicine and, in particular, in surgery and resuscitation practice. For example, cerebral ischemia often accompanies operations on the carotid and vertebral arteries and arch of the aorta, and cardiac arrest when artificial circulation systems are used [3-5, 10]. Mortality among resuscitated patients after clinical death still remains very high (25-30%), and the main cause of this is cerebral in character [8, 9].

For the prevention and treatment of ischemic brain damage when total cerebral ischemia is present, we have used a new Soviet water-soluble form of aspirin, namely acelizine (All-Union Research Institute of Chemical Technology for Therapeutic Substances, Khar'kov).

Clinical trials of acelizine on patients with incomplete myocardial ischemia, i.e., with unstable angina, with primary and repeated myocardial infarction, have shown a therapeutic effect consisting of a decrease in the number of repeated infarcts [1, 2, 7]. It is not known, however, whether acelizine exerts a beneficial effect in total cerebral ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 210 Wistar rats weighing 200-250 g. Cerebral ischemia was created under general intraperitoneal anesthesia [200 mg ketamine (Calipsol)/kg body weight] by the technique developed by ourselves [6]. Acelizine for injections was injected intramuscularly into the animal's right thigh in 1 ml of physiological saline.

There were three groups of experiments. The aim of the first group was to determine whether acelizine can be used to protect the brain against ischemic damage. For this purpose, on a model of 17-min total cerebral ischemia, acelizine was injected 30 min before the beginning of reperfusion in a dose of 25, 50, 100, 150, 200, and 250 mg/kg, calculated as acetylsalicylic acid (ASA).

In the second group the anti-ischemic effect of optimal doses of acelizine, determined previously, was studied after longer periods (25, 30, and 35 min) of cerebral ischemia.

The aim of the third group was to find optimal doses of acelizine for injection. These experiments were carried out on a model of cerebral ischemia with a duration of 25 min, when an optimal dose of the drug was injected 5, 15, 30, and 60 min before creation of ischemia, 15 min before reperfusion, and immediately after reperfusion.

In the early reperfusion period the time of appearance of the response to nociceptive stimulation with forceps, of spontaneous respiratory movements, and of seizure activity was recorded. The number of surviving rats was noted after 2, 4, 12, and 24 h and 2, 3, 4, 5, 6, and 7 days. The clinical manifestations and behavior of the animals were assessed in the early and late stages of the postischemic period. Numerical results were subjected to statistical analysis by Student's test.

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 0007-4888/92/0002-0202$12.50

TABLE 2. Time of Recovery of Nociceptive Sensation, Spontaneous Respiration, and Appearance of Seizure Activity (in min) in Animals Surviving Cerebral Ischemia for 25 and 30 min and Receiving 100 and 150 mg/kg Acelizine

Parameters	Control	Dose of acelizine (mg/kg of ASA)					
		25	50	100	150	200	250
Nociceptive sensation	32,1±1,6	32,8±2,4	26,0±2,8	17,4±1,3**	14,5±1,5**	31,6±4,1	23,4±4,3*
n	18	10	10	10	10	10	10
Spontaneous respiration	75,2±6,6	50,0±6,3*	47,2±4,6*	40,8±3,6**	29,3±2,8**	47,2±5,1*	46,7±7,3*
n	20	9	10	10	10	6	9
Seizure activity	88,8±5,6	69,1±4,3*	58,5±4,1*	60,8±5,1*	49,6±2,4**	—	—
n	15	10	10	10	5		

Legend *) Significance of differences from control ($p < 0.05$), **) significance of differences from control ($p < 0.01$); n) number of experiments.

TABLE 2. Time of Recovery of Nociceptive Sensation, Spontaneous Respiration, and Appearance of Seizure Activity (in min) in Animals Surviving Cerebral Ischemia for 25 and 30 min and Receiving 100 and 150 mg/kg Acelizine

Parameters	Ischemia for 25 min			Ischemia for 30 min		
	control	100 mg/kg	150 mg/kg	control	100 mg/kg	150 mg/kg
Nociceptive sensation	41,67±2,99	20±2,7**	18,9±4,86**	—	23,2±3,15**	22,6±2,1**
n	15	8	8		11	9
Spontaneous respiration	78,33±8,26	44,4±3,19**	50±2,19**	—	54,5±3,49**	57,3±7,95**
n	15	8	7		8	6
Seizure activity	90,8±9,28	60±7,58*	65±4,18*	—	74,3±6,77**	90,7±11,81**
n	11	5	5		8	6

Legend *) Significance of differences from control ($p < 0.05$), **) significance of differences from control ($p < 0.01$); n) number of experiments.

EXPERIMENTAL RESULTS

The use of virtually all doses of acelizine led to marked hyperemia of the skin over the shoulder girdle and visible mucous membranes immediately after the beginning of reperfusion and to the earlier recovery of nociceptive sensitivity, of spontaneous breathing, and seizure activity. The earliest recovery of these parameters was observed when acelizine was given in doses of 100 and 150 mg/kg (Table 1). When acelizine was used in doses of 200 and 250 mg/kg no seizure activity developed.

Assessment of the survival rate of the animals showed that acelizine in doses of 25-150 mg/kg increased the percentage of surviving animals, in a dose of 200 mg/kg it did not affect survival, but in a dose of 250 mg/kg it accelerated death of the animals (Fig. 1).

In the 2nd group of experiments, in which the duration of cerebral ischemia was prolonged to 25 and 30 min, acelizine in doses of 100 and 150 mg/kg led to a significant decrease in the times of recovery of nociceptive sensation and spontaneous respiration and of appearance of seizure activity compared with the control. After cerebral ischemia for 35 min, the physiological parameters were not restored in animals of either the control or the experimental series. No significant differences could be found in the rate of recovery of the vitally important functions of the experimental animals between series receiving 100 and 150 mg/kg acelizine (Table 2).

Analysis of the survival time of the animals showed that death of virtually all the animals in the control series took place within 24 h after the end of a 25-min period of cerebral ischemia, within a few hours after ischemia for 35 min, and 1-1.5 h after ischemia for 35 min. In the experiments with 30-min ischemia acelizine in a dose of 100 mg/kg significantly improved the survival rate of the animals compared with the control in the first 12 h, whereas in a dose of 150 mg/kg it was similarly effective during 3 days of postischemic period (Fig. 2). Analysis of the results of the 3rd group of experiments shows that with all periods of prophylactic administration of acelizine and when it was given 15 min before reperfusion, nociceptive sensation, spontaneous respiration, and seizure activity all appeared significantly faster than in the control. The best results were obtained after injection of the optimal dose of acelizine (150 mg/kg) 30 min before creation of ischemia. Injection of acelizine immediately after removal of the ligatures (beginning of reperfusion) has no effect on the time of

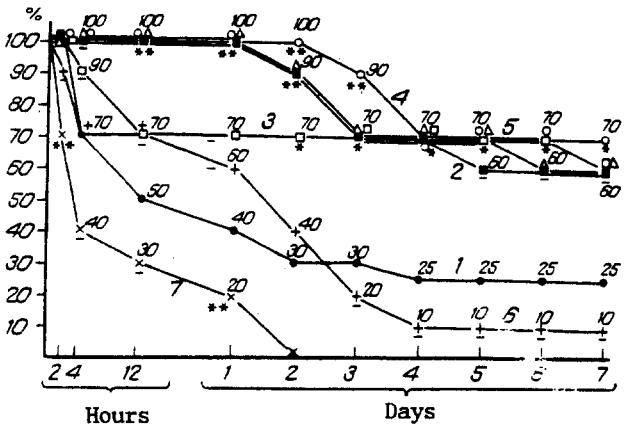

Fig. 1. Time course of survival of rats after total cerebral ischemia and receiving different doses of acelizine (%). 1) Not taking acelizine (control); 2-7) taking acelizine: 2) 25 mg/kg, 3) 50 mg/kg, 4) 100 mg/kg, 5) 150 mg/kg, 6) 200 mg/kg, 7) 250 mg/kg. *) Significance of differences from control ($p < 0.05$), **) significance of differences from control ($p < 0.01$).

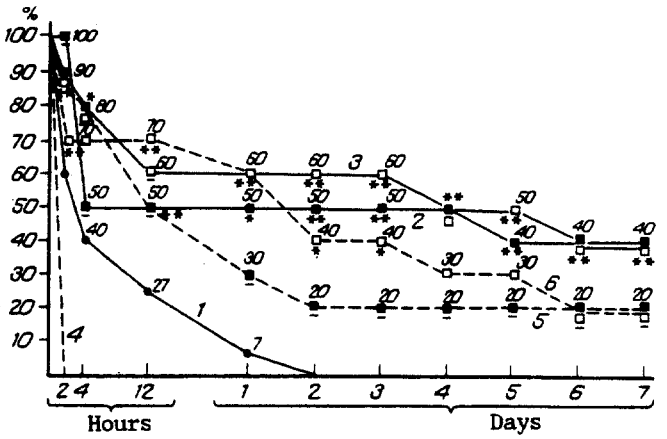

Fig. 2. Time course of survival of rats after total cerebral ischemia for 25 and 30 min, and receiving acelizine in doses of 100 and 150 mg/kg (%). Continuous line – duration of ischemia 25 min; 1) not receiving acelizine; 2, 3) receiving acelizine: 2) 100 mg/kg, 3) 150 mg/kg. Broken line – duration of ischemia 30 min; 4) not receiving acelizine; 5, 6) receiving acelizine: 5) 100 mg/kg, 6) 150 mg/kg. *) Significance of differences from control ($p < 0.05$), **) significance of differences from control ($p < 0.01$).

recovery of spontaneous breathing. In these experiments seizure activity could not be recorded, and the time of appearance of nociceptive sensation was somewhat reduced (Table 3).

The highest survival rate of the animals, namely 80-100%, differed significantly at all times of observation from the control experiments, and it was noted in experiments with injection of acelizine 30 min before the creation of ischemia, After prophylactic administration of acelizine at other times the percentage of animals surviving 7 days was smaller, although compared with the control experiments it was statistically significantly greater. When acelizine was given 15 min before reperfusion, no significant differences in the survival rate of the animals compared with the control were found in

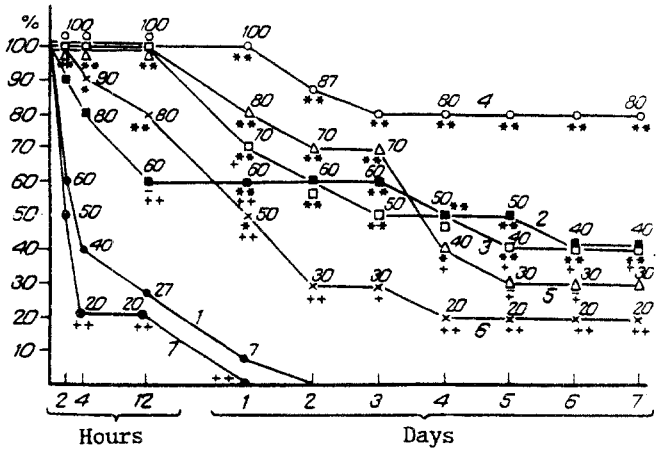

Fig. 3. Time course of survival of rats after total cerebral ischemia for 25 min and receiving 150 mg/kg acelizine at various times before induction of ischemia (%). 1) Not receiving acelizine (control); 2-7) receiving acelizine: 2) 5 min, 3) 15 min 4) 30 min, 5) 60 min before ischemia, 6) 15 min before reperfusion, 7) immediately after beginning of reperfusion. *) Significance of differences from control ($p < 0.05$), **) significance of differences from control ($p < 0.01$), +) significance of differences from 4 series ($p < 0.05$), ++) significance of differences from 4 series ($p < 0.01$).

TABLE 3. Time Taken for Recovery of Nociceptive Sensation, Spontaneous Respiration, and Seizure Activity (in min) in Animals Surviving Cerebral Ischemia for 25 min and Receiving Injection of 150 mg/kg of Acelizine at Different Times before Creation of Ischemia and also 15 min before Reperfusion and Immediately after Beginning of Reperfusion

Parameters	Time of injection of preparation and series No.						
	control	5 min	15 min	30 min	60 min	15 min before reperfusion	after beginning of reperfusion
Nociceptive sensation	41.67±2.99	18.9±1.87**,++	19.88±1.2**,++	9.9±1.06**	17.25±1.81**,++	17.5±2.65**,++	31.8±3.56*,++
n	15	8	9	14	8	8	8
Spontaneous respiration	78.33±8.26	50.0±2.19**,+	31.5±4.27**	39.44±4.54**	34.25±2.97**	38.5±14.25**	88.2±9.34++
n	15	7	9	14	8	8	8
Seizure activity	90.8±9.28	65.0±4.18**,+	41.5±4.4**	48.5±4.07**	52.5±2.4**	55.4±12.41	–
n	11	5	8	13	7	7	

Legend. Significant differences: *$p < 0.05$ with control, **$p < 0.01$ with control, $p < 0.05$ with series 4, ++$p < 0.01$ with series 4; n) number of experiments.

the later stages of observation, but the results of injection of the drug at the beginning of the reperfusion period were similar to the control (Fig. 3).

The animals died mainly when exhibiting hyperreactive motor responses of nonseizure type. In series with injection of acelizine 30 min before the creation of ischemia, disturbances of the neurologic status were more marked and were manifested as transient spontaneous unilateral or bilateral uncoordinated stepping movements.

This investigation thus showed that the Russian preparation acelizine for injection possesses marked anti-ischemic activity against generalized total cerebral ischemia. The best results are obtained if acelizine is injected in a dose of 150 mg/kg 30 min before the beginning of ischemia. This lose has a positive action in the postischemic period – if injected 15 min before the beginning of reperfusion.

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FORMATION OF A STABLE CHLORAMINE COMPLEX DURING INTERACTION OF CARNOSINE WITH THE HYPOCHLORITE ANION

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UDC 615.31:547.96].015.11.07

KEY WORDS: carnosine; sodium hypochlorite; taurine; chloramine.

The dipeptide β-histidyl-L-alanine (carnosine) is nowadays regarded as a new therapeutic agent [10-12] with the rediscovery of its therapeutic properties, which were demonstrated by Soviet scientists as long ago as in the 1930s or 1940s [1, 3-5]. The wound healing, anticataract, and other therapeutic effects of the dipeptide may perhaps be due to its ability to interact with active forms of oxygen. The antioxidative properties of carnosine have been established in a number of studies [6, 10, 13, 14]. For instance, the present writers showed that carnosine can suppress chemiluminescence in the NaClO + H_2O_2 system [7], which can be only partly explained by its ability to quench singlet oxygen [8, 13].

Some free amino acids undergo oxidation by the hypochlorite anion with the formation of unstable chloramines [15]. No information of this kind is available for carnosine. The aim of the present investigation was therefore to study interaction between carnosine and the ClO^- ion.

EXPERIMENTAL METHOD

Solutions of carnosine preparations obtained by organic synthesis (from "Serva" and "Sigma," USA) and by extraction from beef (Leningrad Medical Preparations Factory) were used. Other test objects included solutions of L-alanine and L-histidine ("Serva," USA). A solution of glutathione ("Reanal," Hungary) was interesting as a sulfur-containing compound interacting actively with oxidizing agents, and taurine ("Serva," USA) as a substance forming a stable complex on interaction

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(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. A. Vladimirov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 2, pp. 159-161, February, 1992. Original article submitted May 22, 1991.

0007-4888/92/0002-0206$12.50