METHODS

A Model of Cerebral Circulation Disorders Created by Staged Ligation of the Common Carotid Arteries

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A model of brain ischemia induced by staged ligation of the left and right common carotid arteries has been developed in experiments on rats. The use to this model led to reduction of animal mortality. On days 2-5 after the second ligature, the animals lost weight, the level of their CNS vulnerability increased, the volume of perceived information reduced, adaptation to environmental conditions and reproduction of conditioned reflexes were disordered. Focal and diffuse destructive changes in the nerve and glia cells were found in the cerebral cortex, hippocampus, and thalamic nuclei. The severity of disorders in the blood supply to the brain depended on the interval between ligation of the carotid arteries. This recommends this model for evaluation of the efficiency of drugs of various pharmacological groups.

Key Words: *staged ligation of common carotid arteries; cerebral circulation disorders*

Ischemic stroke is one of the most severe neurological conditions eventuating in death [3,7,8]. One of the numerous models of cerebral ischemia used for evaluation of drug efficiencies is ligation of the common carotid arteries leading to the formation of cerebral circulation reduction similar to ischemic stroke [6,9]. According to some reports, simultaneous bilateral ligation of the common carotid arteries in rats leads to death of 70-100% experimental animals mainly during the first 2-3 days of observation, this impeding studies of the time course of recovery and evaluation of therapy efficiency [2,4,5,10,11].

We evaluated criteria and methods for induction of cerebral circulation disorders by staged ligation of the common carotid arteries for preclinical studies of drug efficiency.

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MATERIALS AND METHODS

The studies were carried out on 90 outbred male albino rats (180-360 g). Three experimental series were carried out, each on 3 groups of 10 animals (intact, sham-operated, and experimental). Cerebral circulation disorders were induced in experimental animals by staged ligation of both common carotid arteries. The intervention was carried out on ether-narcotized animals. At stage 1, the tissues in the left common carotid artery (LCCA) projection were dissected layer-by-layer through an oblique access to the left of the median line, the artery was mobilized 3-4 mm below bifurcation and ligated, after which the wound was treated with an antiseptic (chlorohexidin) solution and sutured through all layers. The wound was treated with 70% ethanol. At stage 2, the right common carotid artery (RCCA) was ligated similarly. The interval between ligatures of the common carotid arteries in

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experimental series I, II, and III was was 1, 2, and 3 days, respectively. Sham-operated animals were subjected to a sham operation (opening of soft tissues, mobilization of the carotid arteries and their elevation by the ligature thread without ligation, soft tissue suturing) under similar conditions.

The status of the animals was evaluated by changes in their body weights and by the number of survivors through 7 days of observation.

The level of orientation and exploratory activity of rats after cerebral ischemia was evaluated in the open field test on days 4 and 7 after the second ligature. Disorders in conditioned reflexes were evaluated by conditioned passive avoidance test (B. I. Lyubimov's modification). The reflex was trained on day 4 after the second operation. The latency of passage from the light to the dark compartment was recorded in the test. Passive avoidance retrieval was evaluated over time on days 7, 11, and 14 after the second operation. The choice of methods used for evaluation of animal status after experimental cerebral ischemia was based on previously published data [1].

Morphological studies were carried out on day 14 after the second ligature. Brain tissues were embedded in paraffin and routinely stained with hematoxylin and eosin. The data were processed statistically using Student's *t* test.

RESULTS

No animal deaths after the first ligature were recorded in the intact, sham-operated, and experimental groups. The time course of mortality in experimental groups is presented for a period of 7 days after the second ligature (Table 1).

Analysis of body weight changes in intact and sham-operated animals showed an increase of this parameter. By day 14 of the experiment it was 10-15% higher than initially starting from day 2 after the second ligature in experimental groups. After the first ligature (LCCA), the experimental animals developed body weight loss of 0.5-5% during day 1. After the second ligature (RCCA) the mean body weight loss during 24 h was 4.5-10%, followed by subsequent reduction of the parameter. In experimental series I, the maximum body weight loss was recorded on days 3-4, in series II on days 4-5, and in series III on day 3 after the second intervention. Stabilization and increase of this parameter to 90-95% of initial body weight was recorded only on day 14 of the experiment. The maximum body weight loss in different series was 15-25%.

Survivors after brain injury (experimental groups in series II and III) exhibited inhibited mobility and disorders in coordination, which made it difficult to evaluate their behavior in the open field test. The majority of the operated rats did not leave the center of the field. A similar picture was observed with passive avoidance performance. Placed into a light box, the animals were in fact motionless, and it was therefore impossible to evaluate the retention of trained reflex. These facts and high mortality early after the intervention, as well as a significant loss of body weight suggested choosing the experimental model with a 3-day interval between the carotid arteries ligature.

Animals with cerebral circulation disorders developed pronounced disorders in the total motor activity by day 4 after the second ligature (Table 2). Horizontal (number of crossed squares) and vertical (number of rearing episodes) components of behavior and the orientation activity level (number of turns) increased significantly. The percentage of passive behavioral activity reduced significantly. These changes seemed to indicate the development of motor anxiety close to the arousal state in the operated on rats. In some animals the number of crossed squares varied from 70 to 131, the number of rearing episodes reached 20-27. Frequent urination was observed in virtually all operated rats.

On day 7 after the second ligature the motor activity of rats with circulatory disorders reduced and did not differ from that in sham-operated animals. However, a significant increase in the parameters characterizing the level of orientation behavior persisted in experimental rats, indicating persistent excitability of the CNS.

TABLE 1. Time Course of Rat Mortality in Experimental Groups after Second Ligature

Experimental series	Day of observation							Number of survivors/ total number
	1	2	3	4	5	6	7	
Series I (1 day between ligatures)	3	1	1	-	-	-	1	4/10
Series II (2 days between ligatures)	3	-	-	-	-	1	1	5/10
Series III (3 days between ligatures)	-	-	-	-	-	-	-	10/10

TABLE 2. Effects of Experimental Cerebral Ischemia Induced by Staged Ligation of Common Carotid Arteries at 3-Day Interval on the Rat Behavior in the Open Field Test (*M*±*m*)

Animal group	Day of observation	Motor activity	Orientation behavior (number of turns)	Passive behavior (duration of immobility periods, sec)	
				number of crossed squares	number of rearing episodes
Intact (*n*=10)	4	12.6±3.9	2.80±0.94	5.8±1.3	207.6±23.9
	7	-	-	-	-
Sham-operated (*n*=10)	4	14.3±1.7	4.50±1.26	5.8±1.2	202.7±14.3
	7	9.0±3.0	6.00±2.66	4.0±0.8	233.4±14.1
Experimental (*n*=10)	4	42.8±12.0*	12.80±2.73*	15.4±2.4*	127.5±22.8*
	7	18.6±3.9	9.1±1.70	8.8±1.7*	187.8±17.5

Note. *$p<0.05$ in comparison with intact and sham-operated animals during the corresponding period of testing.

Ligature of the common carotid arteries at 3-day interval was inessential for the rate of passive avoidance conditioning. On day 4 after the second intervention the reflex was trained in 100% cases. On the other hand, experimental cerebral ischemia modified significantly the process of the reflex reproduction (Table 3).

The data presented in Table 3 indicate significant disorders in passive avoidance performance on days 7 and 11 in 80 and 70% of animals with circulatory disorders, respectively. On day 14 no differences between the groups were observed.

Comparative analysis of behavioral reactions in the open field test and passive avoidance performance showed no clear-cut correlation between the changes in the structure of purposeful behavior and deterioration of passive avoidance performance. This fact indicated different effects of cerebral ischemia on the behavior and memory.

Histological study of the brain on day 14 after the second ligature (RCCA) showed diffuse and focal asymmetrical destructive changes with predominant location in the hippocampus (CA1 zone), thalamic nuclei, and median compartments of the neocortex (Fig. 1). Neurodegenerative processes developed in the stroke focus, leading to the death and disappearance of nerve cells and paralleled by an increase in the num-

TABLE 3. Time Course of Passive Avoidance Performance in Rats after Cerebral Ischemia Induced by Ligation at 3-Day Intervals (*M*±*m*)

Animal group	Day of observation	passive avoidance performance	
		time of first venture into dark box, sec	percentage of rats not daring to enter the dark box
Intact (*n*=10)	7	-	100±10
	11	-	100±10
	14	34	90±10
Sham-operated (*n*=10)	7	-	100±10
	11	-	100±10
	14	41.5±1.5	80±13
Experimental (*n*=10)	7	49.90±12.82	20±13*
	11	62.5±22.5	30±15*
	14	57.5±4.5	80±13

Note. *$p<0.05$ in comparison with intact group.

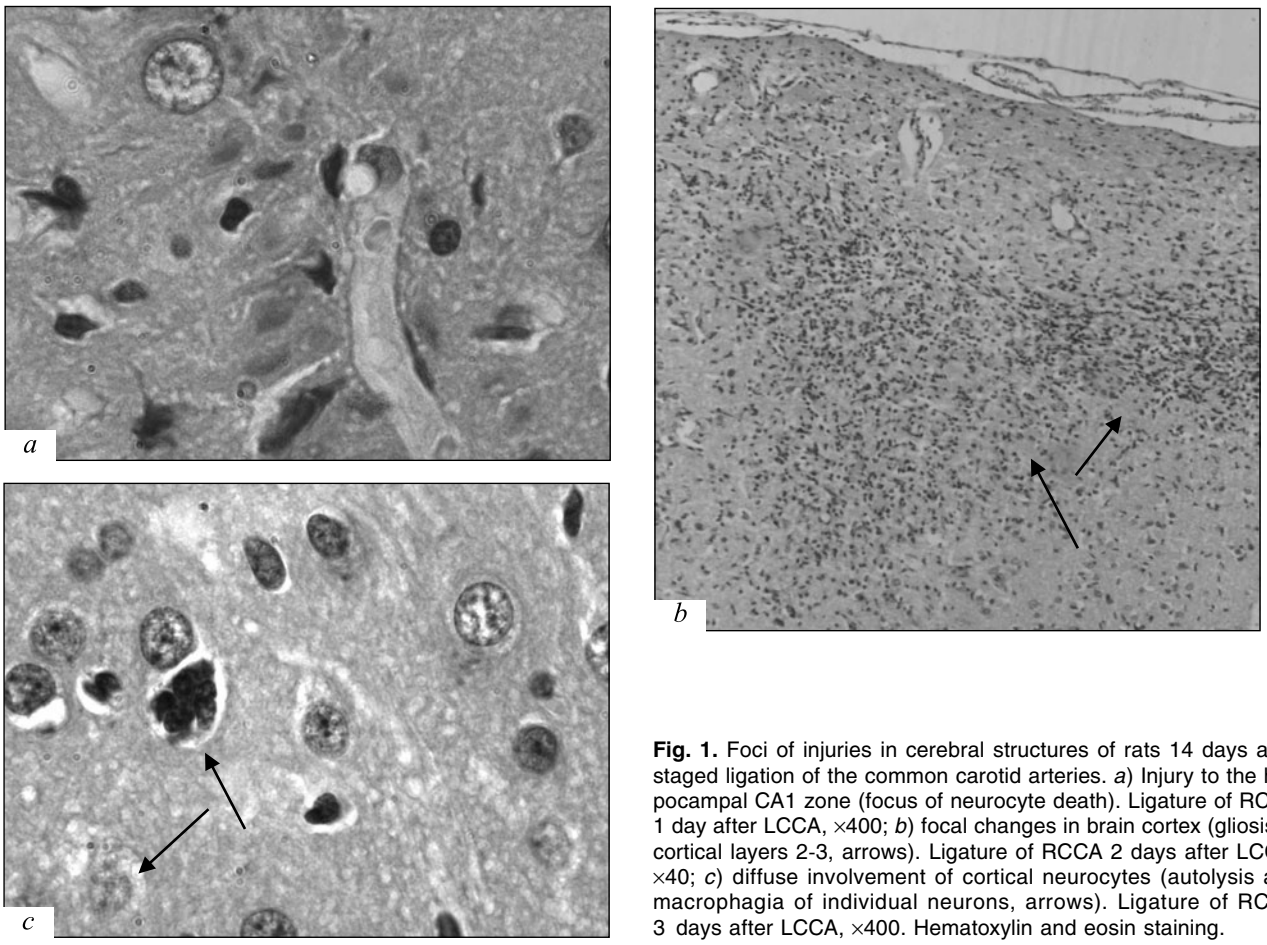

Fig. 1. Foci of injuries in cerebral structures of rats 14 days after staged ligation of the common carotid arteries. *a*) Injury to the hippocampal CA1 zone (focus of neurocyte death). Ligature of RCCA 1 day after LCCA, ×400; *b*) focal changes in brain cortex (gliosis of cortical layers 2-3, arrows). Ligature of RCCA 2 days after LCCA, ×40; *c*) diffuse involvement of cortical neurocytes (autolysis and macrophagia of individual neurons, arrows). Ligature of RCCA 3 days after LCCA, ×400. Hematoxylin and eosin staining.

ber of glia cells. The severity of destructive changes depended on the interval between the interventions: the shorter was the interval, the greater number of brain compartments were involved in the pathological process and the greater were the volume and severity of neurodegenerative changes.

Hence, the results of our studies suggest a method for simulation of incomplete cerebral ischemia in rats, induced by staged ligation of the common carotid arteries. The severity of cerebral circulation disorders depended on the interval between ligation of the carotid arteries. The model of staged ligature with at least a 3-day interval reduced the mortality of animals and allowed the use of behavioral methods for evaluation of their functioning.

Studies on this experimental model showed that cerebral ischemia stimulated the CNS excitability, causing disorders in the behavioral and mnestic functions. It seems that these changes were associated with the formation of focal and diffuse destructive changes in the cerebral cortex, hippocampus, and thalamic nuclei. This model of ischemia is well reproducible and can be used for studies of the mechanisms of cerebral ischemia and evaluation of the efficiency of drugs of various pharmacological groups.

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