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Monitoring of Neurological Deficit and Disturbances in Higher Nervous Activity in Rats with Focal Cerebral Ischemia

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Neurological, locomotor, and behavioral changes in 20 Wistar rats with permanent proximal occlusion of the middle cerebral artery and 18 control sham-operated animals were monitored for 2 months at 10-day intervals. Neurological deficit was maximum immediately after occlusion (3.0±0.6 points), then progressively decreased, but did not completely disappear (0.70±0.06 points on day 60). In control animals neurological status returned to normal on days 10-15. The degree of ketamine-induced rotational asymmetry was 4.0±0.7 rpm over 40 days after surgery and decreased to 2.5±0.6 rpm on day 60. In control rats this parameter only transiently increased to 1.00±0.03 rpm (t_δ=2.5, p<0.05). The time of stay on a rotarod and the latency of passive avoidance in rats with focal cerebral ischemia were lower than in control animals throughout the experiment. The results of complex tests can be used in the experimental search for new drugs for the therapy and rehabilitation of stroke patients.

Key Words: *occlusion of middle cerebral artery in rats; neurological, locomotor, and behavioral deficit*

Stroke is the second leading cause of death in Russia and the most common cause of disability even in young people. Low efficiency of primary neuroprotective therapy and growing number of individuals with persistent functional disturbances in the central nervous system (CNS) necessitate the search for new methods for rehabilitation of stroke patients [6]. The progress in this field is directly related to new advances in experimental modeling of stroke and objective evaluation and monitoring of pathological changes in the late poststroke period.

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Middle cerebral artery occlusion (MCAO) is widely used for modeling of focal cerebral ischemia in rats. This intervention leads to the development of infarction in the basal ganglia, somatosensory cortex, and limbic structures (entorhinal cortex) [4]. There are ambiguous data on sensorimotor disturbances in rats after MCAO. Some authors observed transient (2-3 weeks), while others persistent locomotor disturbances. Surgeries performed by the same technique often produce different results [3,5,8]. It should be emphasized that the duration of testing in these experiments did not exceed 4 weeks, and locomotor and neurological deficit in rats with MCAO was evaluated subjectively by the standard semiquantitative method (neurological score). These peculiarities made difficult comparative analysis of the results obtained in different experiments.

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Here we developed and tested the method for monitoring of the central nervous functions over 2 months after permanent proximal occlusion of the middle cerebral artery in rats. This period is comparable with the chronic stage of ischemic stroke in humans.

MATERIALS AND METHODS

Experiments were performed on 38 adult male Wistar rats weighing 290-310 g. The animals were kept under standard conditions and natural light/dark cycle and had free access to water and food. The rats were weighted at 3-day intervals. The study was performed under ketamine anesthesia (100 mg/kg intraperitoneally).

The rats were divided into two groups: MCAO (n=20) and sham-operation (SMCAO, n=18). Neurological status was determined on days 1, 3, 5, 10, 15, and 20 after surgery and then at 10-day intervals, rotarod performance and ketamine-induced rotational asymmetry were evaluated at 10-day intervals, passive avoidance was tested on days 14, 30, and 60. In 8 rats of each group damage to brain tissue was histomorphologically verified on days 1, 4, 14, 28, and 60 after surgery.

MCAO was modeled on the left middle carotid artery (MCA) without damage to the zygomatic process and facial nerve (method of Tamura with Bederson's modifications) [1]. MCA was electrocoagulated between the point localized 2 mm proximal to *tractus olfactorius* and the intersection between this artery and inferior cerebral vein. The surgical field was covered with a skin flap. SMCAO included all surgical manipulations except for electrocoagulation of MCA.

Neurological status was estimated by the Menzies scale [4] after weighing: no deficit (0 points), tonic flexion of the forelimb contralateral to the side of MCAO during tail suspension (1 point), low resistance of the contralateral forelimb to passive movement during tail stretch on a horizontal surface (2 points), contralateral movement of rats during tail pinch (3 points), and spontaneous contralateral rotation of animals on a horizontal surface (4 points). These points were summarized.

Immediately after neurological examination the rats were tested on a rotating rod (diameter 70 mm, length 200 mm) positioned at a distance of 80 cm from the floor for evaluation of locomotor and coordination disturbances. On day 10 after MCAO or SMCAO the rats were twice tested on a rotating rod (4 rpm, 2×10 min). The procedure of testing was performed in 3 stages: stage 1, 7 rpm; stage 2, 14 rpm; and stage 3, 21 rpm (maximum revolution rate). At each stage the rats were placed on a rotating rod only 1 time. The time of stay on a rotating rod was recorded. The test was stopped if the rats remained on the rod for more than 180 sec (maximum period).

Studies of ketamine-induced rotational asymmetry were performed after the rotarod test. The animals intraperitoneally received 5% ketamine in a dose of 50 mg/kg and were placed in an automatic rotameter. The device equipped with computer software allowed us to estimate the number of leftward and rightward rotations (the difference between left- and rightward rotations per 1 min (K-test) was estimated over 40-min testing).

Passive avoidance behavior (conditional inhibition of moving from a platform) was studied on day 14 and by the end of the 1st and 2nd months after surgery (on days free from other tests; cognitive defi/cit and memory disorders were evaluated. The chamber was a wooden box (35×20×30 cm) with a metal-grid floor. A wooden platform (8×5 cm) was positioned in one corner of the box at a height of 7 cm. During training the rats were placed on a platform. When the animals came down, electric current (0.2 mA) was delivered through a metal grid until they returned to a platform and remained there for 10 min. On the next day the rats were placed on a platform, and the latency of moving down was recorded. Electric current was not applied to the floor. If the rat remained on a platform for 180 sec the test was stopped.

For histomorphological examination the brain was perfused through the ascending aorta with 200 ml phosphate buffered saline and then with 400 ml 4% neutral paraformaldehyde in phosphate buffered saline. Then

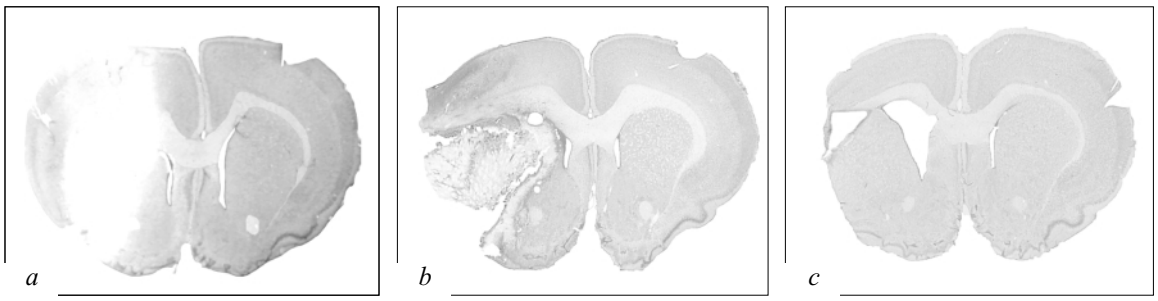

Fig. 1. Morphological signs of focal ischemia in rats at various terms after middle cerebral artery occlusion. Coronary brain sections; staining with cresyl violet; scanning (1600 dpi). Day 1 after surgery, colorless zone of infarction (*a*); day 4, gliomesodermal infiltrate around the zone of pannecrosis (*b*); day 30, cavity in the zone of infarction, enlargement of the lateral ventricle (*c*).

brain was removed and placed in paraformaldehyde of the same concentration. Brain samples were kept in 20% sucrose, serial 20-μ sections were prepared on a freezing microtome. The sections stained with cresyl violet by the method of Nissl were examined under a light microscope and scanned.

The results were analyzed by Student's *t* test and Fischer's *U* test.

RESULTS

None rats died after surgery. The rats with MCAO more slowly gained weight compared to control animals. In rats with focal cerebral ischemia the postoperative body weight loss was observed over the first

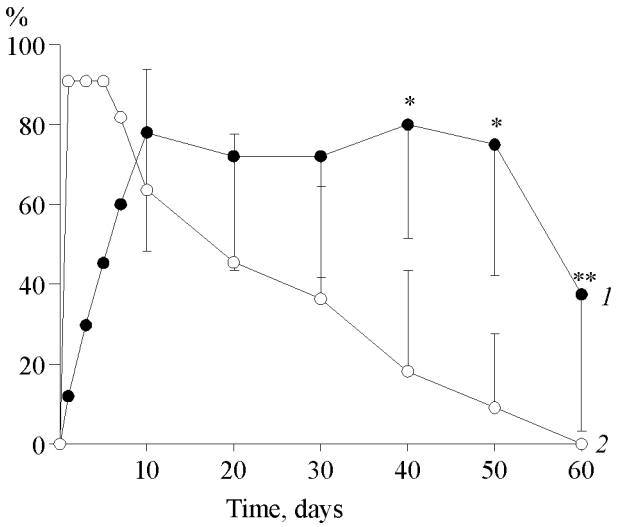

Fig. 2. Ratio of rats (%) with ketamine-induced rotational asymmetry (*1*) detected by neurological examination after middle cerebral artery occlusion (*2*). *$p<0.01$ and **$p<0.05$ compared to *2*.

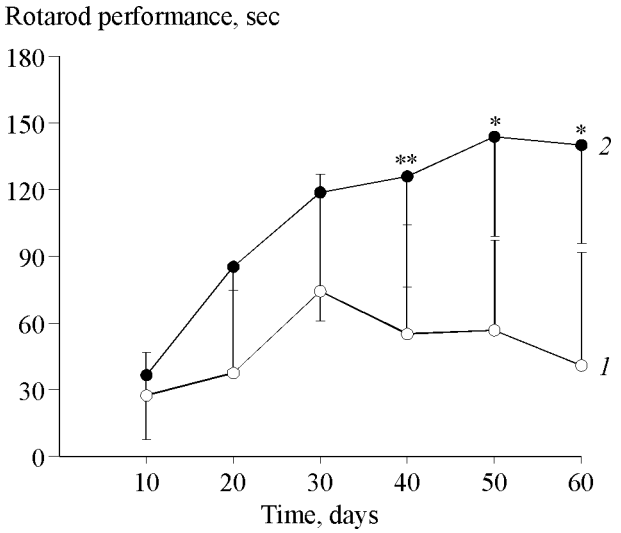

Fig. 3. Time of stay on a rotating rod (14 rpm) after modeling of focal cerebral ischemia (X_m, sec). Occlusion of the middle cerebral artery (*1*); sham-operation (*2*). *$p<0.01$ and **$p<0.05$ compared to *1*.

15 days after surgery, while in controls (SMCAO) body weight returned to normal after 2-3 days. The differences in body weight became significant starting from day 5 after surgery ($p<0.05$) and further increased in the follow-up period. By the end of observations the relative increase in body weight in rats with MCAO and SMCAO was 18.8±12.0 and 55.8±7.5%, respectively ($p<0.01$).

The rats with MCAO had focal cerebral ischemia morphologically manifested in extensive infarction followed by its organization (formation of scar or cavity, Fig. 1). Pannecrosis involved considerable areas of the somatosensory cortex and striatum and spread to limbic structures (primary olfactory and entorhinal cortex) and external capsule (Fig. 1, *a*). Agglomerates of glial cells and leukocytes (gliomesodermal infiltrate) were found around the infarction zone on day 4 after MCAO (Fig. 1, *b*), after 10-14 days we found a small cavity in the zone of pannecrosis and minimal inflammatory changes in the surrounding tissue. On days 28-60 scar formation was accompanied by shrinkage and deformation of structures in hemispheres and dilation of the ventricle at the side of infarction. The ventricle was sometimes connected with the cavity (Fig. 1, *c*). In control rats mild cerebral edema in the projection of trepanation hole were revealed on days 2-3 after SMCAO.

Neurological deficit was maximum immediately after MCAO (3.0±0.6 points), then progressively regressed, but remained high by the end of observations (0.70±0.06 points, day 60). In control animals we revealed only mild neurological disturbances (less than 1 point) that returned to normal on days 10-15.

The rats with focal cerebral ischemia were characterized by ipsilateral rotational asymmetry. K-test in these animals was 4.0±0.7 rpm over 40 days after surgery and decreased to 2.5±0.6 rpm by day 60. In control rats this parameter only transiently increased to 1.00±0.03 rpm (t_δ=2.5, $p<0.05$). A comparison of the ratio of rats with pronounced locomotor asymmetry revealed by K-test (more than 2 rpm) and neurological examination (more than 3 points) showed that K-test was much more sensitive than neurological examination (Fig. 2).

Rotarod performance in rats with MCAO (14 rpm) was poorer than in control animals. These differences progressed over 2 months (Fig. 3). In the MCAO group the ratio of rats staying on a rotating rod for 90, 120, and 180 sec was lower compared to the control (Table 1).

The latencies of moving from a platform in the passive avoidance test was 43±37, 46.9±45.0, and 58±55 sec on days 14, 30, and 60 after MCAO, respectively. In the SMCAO group these latencies were longer (144±41, 125±49, and 144±45 sec, respectively).

TABLE 1. Coordination Disturbances in Rats with Focal Ischemia (%, *M*±*m*)

Time of testing, sec		Time after modeling of focal ischemia, days					
		10	20	30	40	50	60
More than 90	MCAO	0	9±8	30±15	22±14	11±10	12±11
	SMCAO	10±9	50±16**	60±15	60±15	80±13*	80±13*
More than 120	MCAO	0	9±8	30±15	22±14	11±10	12±11
	SMCAO	10±9	30±15	60±15	60±15	70±15**	70±15**
More than 180	MCAO	0	9±8	20±23	11±19	11±10	12±11
	SMCAO	0	20±13	60±15	50±16	70±15**	30±15*

Note. *p<0.01 and **p<0.05 compared to MCAO.

Following recommendations of C. V. Borlongan *et al*. [2], higher nervous activity was tested only after 10 days to exclude the transient postoperative effect of focal cerebral ischemia. Neurological status was determined starting from the day of MCA occlusion. Our results are consistent with published data that neurological deficit in rats with MCAO was completely compensated one month after surgery [2-5]. Therefore, objective loading tests for latent (compensated) pathological changes were used for evaluation of the state of animals in the follow-up period. Locomotor disturbances (rotarod performance and ketamine-induced rotational asymmetry) and cognitive deficit (passive avoidance test) in rats with focal cerebral ischemia persisted for 60 days after MCAO. Changes in body weight are a reliable criterion for the state of animals. In animals with MCAO severe functional disturbances in CNS were accompanied by deceleration of body weight gain compared to the control.

Our results show that the rats with MCAO are characterized by persistent locomotor and cognitive deficit. The tests used in our experiments allowed evaluation of activity of CNS in early and late stages after modeling of focal cerebral ischemia. These data offer considerable scope for experimental studies of medicinal preparations that promote recovery of structures and functions impaired after stroke.

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