METHODS

New Minimally Invasive Model of Spinal Cord Ischemia in Rats

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We developed a new minimally invasive model of spinal cord ischemia in rats: intravascular occlusion of the abdominal aorta and its branches. This model can be used on small laboratory animals and allows qualitatively and quantitatively evaluating the morphofunctional state of the nervous system during spinal cord ischemia by clinical manifestations and histological changes. Selective intravascular occlusion determines minimal invasiveness and adequacy of the proposed model to *in vivo* pathological processes. This model of spinal cord ischemia can be used in experimental pharmacology for evaluation of neuroprotective activity of various drugs and bioactive substances.

Key Words: *model; ischemia; spinal cord; rats*

Ischemic damages to the spinal cord (SC) are an important medical problem [1,4]. Biological studies are performed on various models of SC ischemia. Most models can be used only on large animals or are highly invasive, which introduces errors into measurements. We developed a new minimally invasive model of SC ischemia, which can be used on small laboratory animals [2,3,5,6].

MATERIALS AND METHODS

Experiments were performed on 25 outbred female rats weighing 100-160 g. The animals were narcotized with sodium ethaminal (30 mg/kg).

Transitory ischemia of the lumbar SC was induced by total intravascular occlusion of the abdominal aorta and its branches (Fig. 1). To this end, occluders were introduced into both femoral arteries toward the heart to a distance equal to the distance from the xiphoid to the base of the tail. Feracryl (0.3 ml, 1%; Pusk Company, Irkutsk) was injected into the left common carotid artery below the site of ligation toward occluders. The time of feracryl administration corresponded to the beginning of ischemia. The occluders were removed after 45 min, and femoral arteries were ligated. Sterile chromic catgut threads 3.0 served as occluders.

In group 1 rats (n=10), SC ischemia was modeled by this method. In group 2 rats (n=12) various modifications of this model were tested: one catgut occluder (n=2) or two catgut occluders (n=2) were implanted without feracryl; 1% feracryl was injected without occluders (n=2, 0.4 and 0.5 ml, respectively); one occluder was implanted and 0.3 ml feracryl was injected (n=2); two polypropylene occluders were implanted and 0.3 ml feracryl was injected (n=1); feracryl (>0.3 ml) was injected and one (n=1) or two (n=2) catgut occluders were implanted.

Deep, tactile, and temperature sensitivity were evaluated by a 5-point scale at 5-min intervals: no reaction (0 points), weak reaction to stimulation (1

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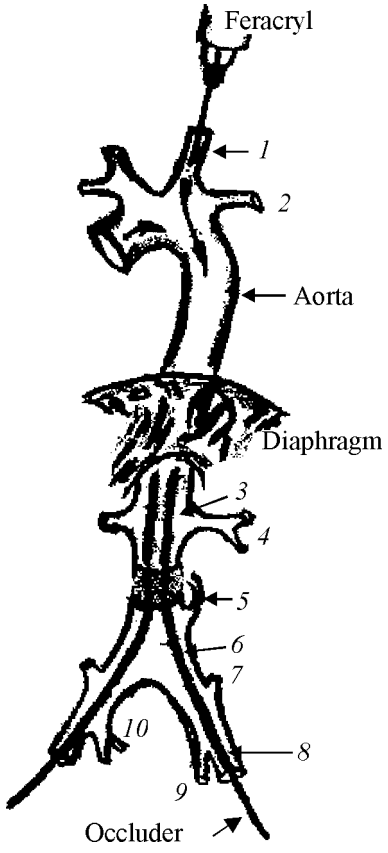

Fig. 1. Implantation of occluders during modeling of intravascular occlusion of the abdominal aorta and its branches in rats: left common carotid artery (*1*), subclavian artery (*2*), Adamkiewicz artery (*3*), renal artery (*4*), 2nd lumbar artery (*5*), common iliac artery (*6*), iliolumbar artery (*7*), external iliac artery (*8*), internal iliac artery (*9*), and lateral sacral artery (*10*).

point), significantly decreased and/or delayed reaction (2 points), slightly decreased and/or delayed reaction (3 points), and unchanged reaction (4 points).

Methylene blue was injected intraarterially to study its distribution in rat arteries under normal (n=1) and ischemic conditions (n=2).

SC was isolated and fixed in 96% alcohol after 48 h. Transverse sections of SC at the level of the lumbar enlargement were stained with hematoxylin and eosin. We counted neurons at various stages of histopathological changes (normal, chromatolytic, and ghost cells).

The results were analyzed by Student's t test.

RESULTS

Ischemia modeled according to the proposed method was accompanied by progressive and symmetric loss of sensations, paleness, coldness, and atony of hindlimb muscles, and decrease in the sensitivity of forelimbs and hindlimbs (from distal to proximal regions). In the postischemic period clinical signs included symmetric peripheral paraplegia developed immediately after surgery, complete loss of sensitivity in hindlimbs and lumbar region, and pelvic disorders. Trophic changes in muscles of the lumbar region and hindlimbs developed 2 days after surgery.

In group 1 rats the response to deep, tactile, and thermal stimuli decreased 18.5±4.3, 15.0±4.2, and 17.5± 5.7 min after surgery, respectively. Practically complete loss of sensations was found after 45-min ischemia (without significant differences between various types of sensations, Fig. 2).

Macroscopic and microscopic examinations revealed ischemic damages to organs of the abdominal cavity, retroperitoneal space, and lumbar region.

Histological examination of the lumbar enlargement revealed considerable ischemic injuries in group 1 rats. Histopathological changes included destruction of fibrous structures, homogenization and chromatolysis of the neuronal cytoplasm, karyopyknosis, disintegration of Nissl bodies, and transformation of neurons into ghost cells. We revealed pronounced neuronophagy and edema of the white matter. Most pronounced changes were observed in the anterior horns of SC (Fig. 3). Normal, chromatolytic, and ghost neurons comprised 29, 17, and 54%, respectively.

In group 2 rats implantation of a single catgut occluder caused no motor and sensory disturbances in hindlimbs. Implantation of two occluders was followed by insignificant motor disturbances in distal hindlimb muscles. Histopathological changes in internal organs and lumbar enlargement of SC were absent. Injection of feracryl alone, administration of feracryl in a dose of above 0.3 ml with implantation of occluders, or use of polypropylene occluders caused animal death within 24 h after treatment. Implan-

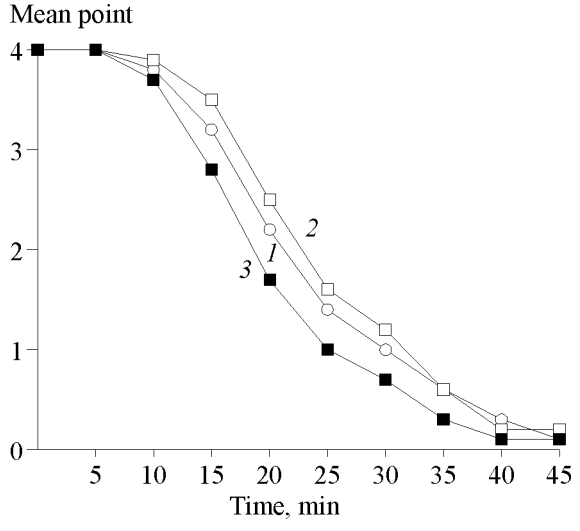

Fig. 2. Loss of temperature (*1*), deep (*2*), and tactile sensitivity (*3*) in rats during SC iscemia modelling.

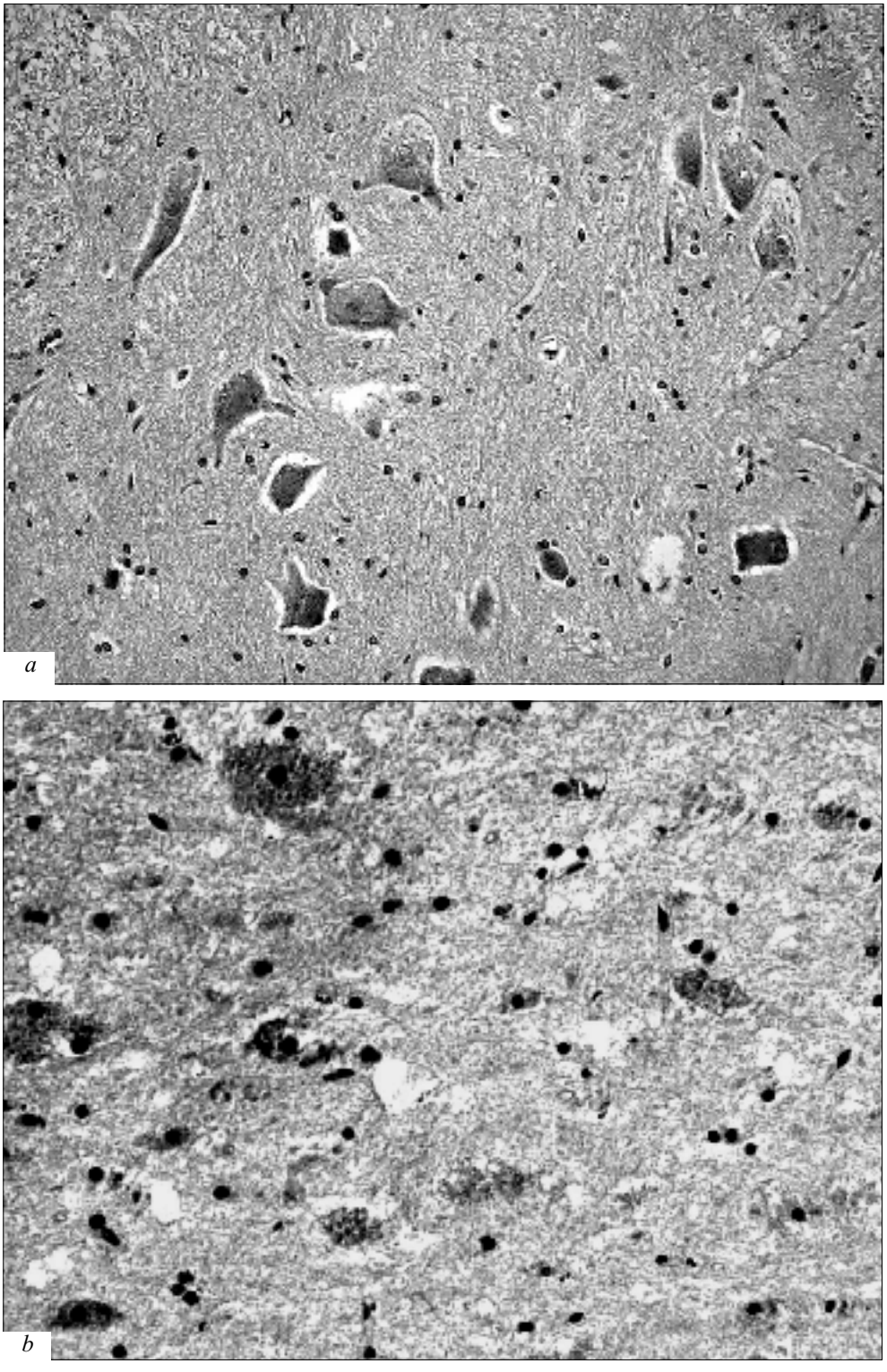

Fig. 3. Histological examination of transverse sections of the spinal cord at the level of the lumbar enlargement under normal conditions (*a*) and during ischemia (*b*). Staining with hematoxylin and eosin (×200).

tation of a single occluder under similar experimental conditions did not led to the development of neurological deficiency and histopathological changes in internal organs and lumbar enlargement of SC. Modeling of SC ischemia without removal of occluders led to animals death within 24 h after treatment.

In control rats injection of methylene blue into the aorta was followed by its regular distribution in

vessels. After modeling of ischemia by the proposed method, the stain was regularly distributed in all branches of the aortic arch and thoracic aorta. Independently on the model of SC ischemia occluders completely blocked the distribution of methylene blue.

Implantation of occluders into the femoral and iliac arteries impairs collateral blood flow through lumbar radicular arteries and anastomotic loop of the SC cone. Precipitation of the protein-feracryl complex (iron-containing salt of polyacrylic acid) on the catgut thread led to thickening of the occluder and obturation of the abdominal aorta without changes in its diameter. Feracryl binds primarily to serum albumins with the formation of viscous rubber-like insoluble complexes. It does not cross biological barriers, possesses no toxic activity, and does not modulate physiological and pathophysiological processes. Previous studies showed that feracryl has no neurotoxic properties [7].

Modeling of ischemia by the proposed method was followed by pronounced ischemic changes in the lumbar enlargement of SC. The distribution of sensory and motor disturbances from distal to proximal regions suggests that ischemia develops from the outside to deep zones, which corresponds to the Auerbach-Flatau rule (eccentric position of long pathways) and peculiarities of blood supply to SC. Severe trophic changes in muscles of hindlimbs and lumbar region were observed 2 days after surgery. They resulted from pronounced disturbances in trophic functions of nerves, which was associated with ischemic damages to the lumbar enlargement and 45-min ischemia of limbs. Tactile sensation disappeared most rapidly, which was probably related to more severe damages to the ventral area of the lumbar enlargement (histologically verified).

In group 2 rats implantation of occluders without administration of 1% feracryl produced motor disorders in the distal limb muscles, which was probably associated with impaired blood flow in lumbar radicular arteries due to occlusion of iliac arteries. Death of animals over the 1st day after injection of feracryl alone (0.3 ml), administration of feracryl in a dose of more than 0.3 ml with and without implantation of occluders, and use of polypropylene occluders was related to embolism of aortic branches with feracryl-albumin clots. After implantation of polypropylene occluders feracryl-blood albumin clots were not fixed on occluders, which caused embolism. Implantation of a single occluder under similar experimental conditions was low effective.

Modeling of ischemia for more than 45 min markedly increased the risk of death due to pronounced functional disturbances in internal organs. To estimate the efficiency of the proposed method, methylene blue was introduced intraarterially under conditions of normal blood supply to SC and after modeling of ischemia. Implanted occluders completely blocked blood flow, which indicates that this model of SC ischemia is highly efficient.

The proposed model of SC ischemia is minimally invasive, requires no special devices and instruments, and allows evaluating the level of descending aorta occlusion and stage of ischemia. The thickness of occluders can be changed and, therefore, this method can be used for small laboratory animals. The proposed method can also be used for modeling ischemia of internal organs. Modeling of SC ischemia by this method leads to severe ischemic injuries in the lumbar enlargement of SC and, therefore, holds much promise for studying pharmacological activity of various drugs and for other biomedical experiments.

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