A New Rat Model of Reversible Global Cerebral Ischemia

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A new rat model of global cerebral ischemia–reperfusion was proposed via reversible occlusion of the major vessels originating from the aortic arch and supplying the brain. This technique can be used for the search and study of exogenous (pharmacological) and endogenous methods of brain protection from ischemia–reperfusion injury.

Key Words: *global ischemia; ischemia—reperfusion; brain; Wistar rats*

Among the leading causes of mortality in the world, acute cerebrovascular disorders rank third after CHD and cancer. In light of this, the search for effective means ameliorating cerebral ischemia–reperfusion injury (CIRI) is one of the major problems of experimental medicine and biology. Global CIRI occurs during resuscitation after sudden cardiac arrest, during perinatal hypoxia, carotid endarterectomy, and often accompanies the cardiac surgery with cardiopulmonary bypass. The studies aimed at finding and study of neuroprotective means against global CIRI are limited by lack of optimal experimental model of reversible ischemia followed by reperfusion in all compartments of the brain of experimental animals.

Two rat models of global cerebral ischemia are now used: two-vessel model with hypotension [7] and four-vessel model [5]. The main drawback of the two-vessel model with hypotension is that systemic hypotension leads to generalized metabolic disorder in vital organs including the brain [3]. In 1979, the four-vessel rat model of global cerebral ischemia was proposed consisting in occlusion of both common carotid arteries and both vertebral arteries. The four-vessel model has several major drawbacks: 1) electrocoagulation of vertebral arteries through *for. alare* in the first cervical vertebra requires deep and traumatic surgical approach associated with considerable damage to muscles; 2) coagulation of vertebral arteries may result in incomplete occlusion; 3) coagulation of vertebral arteries in the majority of experimental animals leads to massive bleeding; 4) the diameter of *for. alare* in the first cervical vertebra varies greatly and is often less than 1 mm, and therefore electrocoagulation cannot be properly performed; 5) ischemia modeling takes two days [1,4,9,11]. Some modifications of the four-vessel model were proposed [8,6,11]. However, they did not eliminate its shortcomings, in particular, massive bleeding after coagulation of vertebral arteries [6] and residual collateral blood flow.

During the last two decades, pre- and postconditioning phenomena were actively studied as the means of brain protection against ischemia–reperfusion injury [2,10]. The protective effect of these phenomena is most pronounced during reversible cerebral ischemia. That is generally consistent with the modern concept of early brain revascularization after ischemic stroke. The mechanisms underlying such endogenous neuroprotective effects as brain pre- and postconditioning can be studied on the models of reversible focal and global ischemia (*i.e.* ischemia followed by reperfusion). It should be noted that the existing four-vessel model of global cerebral ischemia does not meet this requirement, because it includes permanent vertebral basilar ischemia.

The aim of the study was to develop and test the new model of cerebral reversible global ischemia devoid of shortcomings of the previous models.

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MATERIALS AND METHODS

All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23) and were approved by local ethics committee. Experiments were performed on male Wistar rats (n=35) weighing 220-250g (Rappolovo Breeding Center) kept under a 12:12 h light-dark regime and receiving standard laboratory pellets and drinking water *ad libitum*.

The animals were anesthetized with chloral hydrate (450 mg/kg intraperitoneally). During the experiments, the rats were intubated and jet ventilation was performed (SAR830; Stoelting Inc.). An L-shaped skin incision was made on the midline of the chest and then in the axillary region, after that the skin was separated along the section. Similarly, *m. pectoralis major* and *m. pectoralis minor* were separated 1 mm from the midline to the left. This was followed by an incision along the bottom edge of the third rib, allowing the access to the torax. Aortic arch and the major vessels originating from it were separated (*tr. brachiocephalicus, a. subclavia sin., a. carotis communis sin.*). Ligatures were placed under the vessels and then microvascular clips were applied for 15 min for cerebral blood flow arrest. The clips were removed after 15 min and brain perfusion was resumed. The pneumothorax was released and the surgical wound was sutured in layer-by-layers. Thoracic cavity was hermetically sealed with intercostal sutures and the wound was covered with previously separated layers of muscles and skin. Then the L-shaped incisions made in the beginning of the operation were joined by interrupted sutures. The final stage of the operation implied resumption of spontaneous breathing. The mucus was removed from the mouth and trachea, and then artificial ventilation was stopped. Three minutes after appearance of spontaneous breathing, the animal was extubated. Sham-operated animals underwent the same procedures except application of microvascular clips on the main vessels. All surgical procedures were carried out on an operating table thermostatically controlled at 37°C. In the post-operative period before exit from anesthesia, core temperature was also maintained constant by an external heat source.

Survival was evaluated 24 and 48 h after surgery. After 48 hours, the survivors were narcotized and decapitated. For histological examination, frontal brain sections were stained with hematoxylin and eosin. For additional analysis of cerebral blood flow, 1% Evans blue was injected *in vivo* into the femoral vein after clamping the vessels originating from the heart and without clamping. In addition, the carotid and vertebral arteries were punctuated under the same conditions.

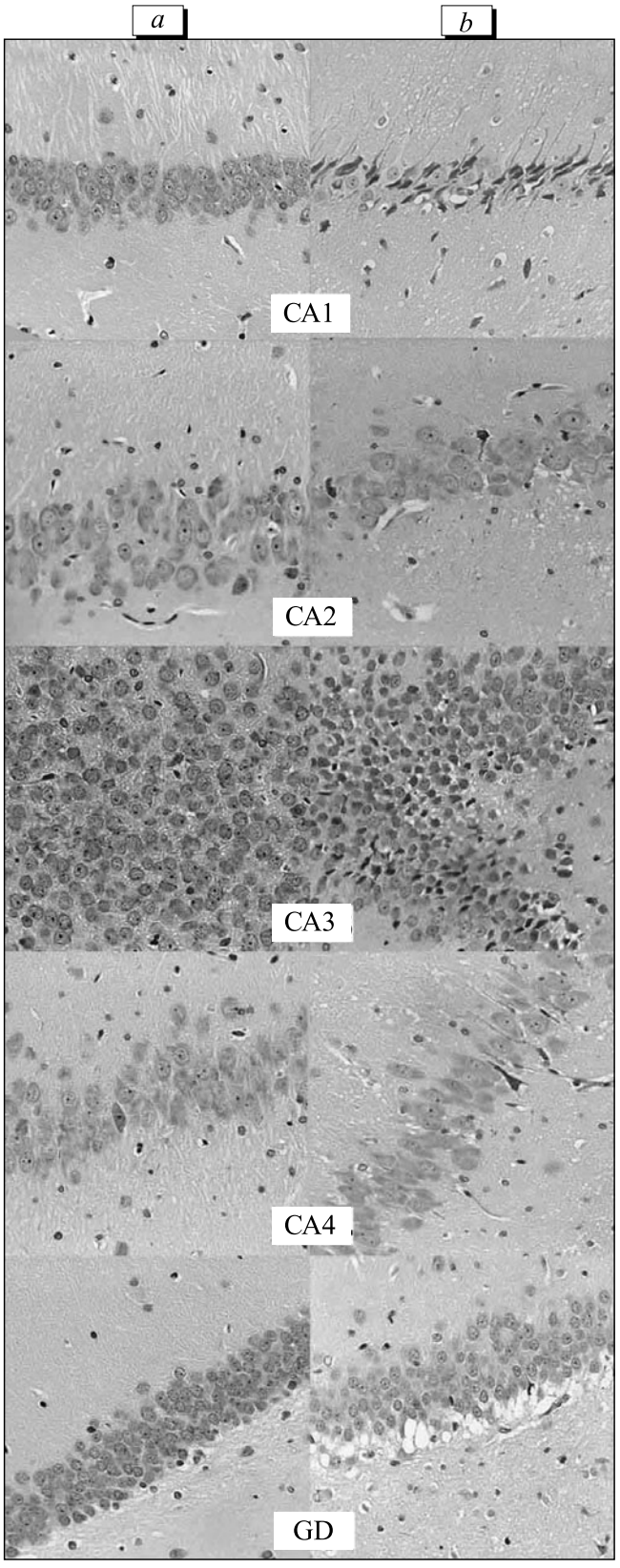

Fig. 1. Zones of rat hippocampus 48 hours after sham operation (*a*) and 15-min global ischemia (*b*). Hematoxylin and eosin staining, ×400. Normal hippocampal pyramidal cells have round pale nuclei, while necrotic and necrobiotic cells have pyknotic nuclei.

TABLE 1. Survival of Rats after 15-min Reversible Global Brain Ischemia Modeled by the Proposed Method

Group	Animal survival	
	day 1 after surgery	day 2 after surgery
Sham operation (n=9)	88.9% (8/1)	88.9% (8/1)
Global ischemia (n=12)	75.0% (9/3)	41.7%* (5/7)

Note. Survivors/dead animals ratio is shown in parentheses. *P=0.0375.

Reliability of differences in animal survival was assessed by Fisher's exact test using Statistica 6.0 software. The differences were significant at $P<0.05$.

RESULTS

In 5 rats, both carotid arteries were dissected after clamping the major vessels before punctation of *a. vertebralis* via *for. alare* in the first cervical vertebra. The major vessels did not bleed during clamping, but after clamp removal severe bleeding from the carotid and vertebral arteries was observed in all 5 animals. The second series of additional experiments included injection of 1.5 ml 1% Evans blue into the femoral vein. When the major vessels were clamped, the brain and tissues lining the inner surface of the skull remained unstained (n=5) in contrast to the experiments without artery occlusion (n=4).

The data on animal survival after global CIRI modeling according to the proposed protocol are presented in Table 1. Survival of animals with global ischemia on postoperation day 1 did not differ significantly from that of sham-operated controls ($P>0.05$). However, survival in the group with global ischemia by day was significantly lower than in sham-operated animals (41.7%, P=0.0375). The increase in animal mortality by day 2 after 15-min ischemia modeling can be explained by delayed neuronal death. Histological examination of frontal brain sections revealed alterations in cells of the cortex, striatum, and hippocampus: perivascular and pericellular edema, damage to all zones of the hippocampus, primarily CA1 and CA3 pyramidal neurons, accompanied by cell shrinkage, swelling, hyperchromatosis, and chromatolysis (Fig. 1). Hippocampus and especially CA3 and CA1 zones are most sensitive to ischemia [5,10].

Thus, we propose a new method for modeling reversible global cerebral ischemia tested experimentally on rats. It produces ischemic and reperfusion brain injury documented both experimentally *in vivo* and by histological examination. The proposed model is reproducible and ensures high animal survival after surgery; the experiments can be carried out on different strains of rats and on animals with different body weight. The proposed model can be used to adequately simulate such clinical events as cardiac arrest followed by resuscitation, asphyxia, cardiac surgery with cardiopulmonary bypass, surgical procedures on arteries of the neck. The proposed model extends our knowledge on the mechanisms underlying cerebral global ischemia and allows identification of cerebral areas and structures most vulnerable to global ischemia as well as zones of hypoperfusion formed after blood flow resumption. The model allows us to explore the mechanisms underlying exogenous (pharmacological) and endogenous neuroprotectivion including ischemic pre- and postconditioning and to develop recommendations for clinical decision support.

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