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An improved three-vessel occlusion model of global cerebral ischemia in rats

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ABSTRACT

We developed an improved three-vessel occlusion model of global cerebral ischemia in rats. This method consists in cessation of cerebral blood flow by accessing *a. carotis communis sinistra* through the ventral surface of the neck as well as *tr. brachiocephalicus* and *a. subclavia sinistra* through the first intercostal space, bypassing the pleural cavity and excluding pneumothorax. After the occlusion of the vessels that resulted in interruption of their blood flow, according to laser-Doppler flowmetry, there was a sharp decline in local cerebral blood flow in the visual cortex to 4 ± 1% of the initial level. After restoring the level of local cerebral blood flow at the 5th minute, 10th minute, 20th minute and 24th hour of reperfusion, the levels of local cerebral blood flow were 51 ± 7%, 41 ± 5%, 35 ± 8% and 54 ± 9% of the initial level, respectively. Histo-quantitative analysis of changes in neurons of the hippocampus of rats showed that after ischemic injury, the numerical density of neurons in hippocampal zone CA1 in the observed 1 mm^2 region decreased by 29%, 22%, and 35%, respectively, compared to sham-operated animals ($p < 0.05$). By the first day after global cerebral ischemia, the experimental group had shown a mean neurological deficit score equal to 7.5 ± 1.0 and 7.9 ± 0.7 points, followed by a decrease up to score 6.5 ± 1.1 and 5.9 ± 0.7 on the third day, 4.6 ± 0.8 and 4.7 ± 0.5 on the fifth day (on chloral hydrate and propofol anesthesia correspondently).

1. Introduction

Global ischemic brain injury is a leading cause of death after cardiac arrest episodes. In-hospital mortality among successfully reanimated patients is 70%, while in 2/3 of surviving patients, a moderate or severe neurological deficit develops within 3 months after a cardiac arrest episode (Mangus et al., 2014). Even a short disruption of cerebral blood supply leads to severe brain damage (Flynn et al., 2008) and irreversible cognitive and executive function impairments (Karanjia and Geocadin, 2011).

Models of global cerebral ischemia (GCI) are usually used to study brain damage that occurs during cardio-circulatory resuscitation. Modeling GCI in experimental animals stops the blood flow in the main blood vessels supplying the brain (Richard Green et al., 2003). The most frequently used method for reproducing GCI is four-vessel occlusion (4VO), which was developed by W.A. Pulsinelli (Pulsinelli and Brierley, 1979). However, 4VO is associated with a number of serious technical problems, including difficulty of electrocoagulation of the vertebral arteries through the alar foramina of the first cervical vertebra, risk of spinal cord damage, and risk of excess bleeding (Chen, 2009; Ohnishi and Ohnishi, 1995; Pulsinelli and Buchan, 1988), a preconditioning effect (Yamaguchi et al., 2005), incomplete ischemia of the brain stem (Pulsinelli and Brierley, 1979). As an alternative to the 4VO model, Shcherbak et al. (2012) proposed a new way of performing GCI modeling in rats by reversible occlusion of the three main vessels that branch off from the aorta and supply the brain (brachiocephalic trunk, left subclavian artery, and left common carotid artery). The advantages of this three-vessel occlusion (3VO) model consist in the possibility of performing a one-step operation and absence of residual collateral blood flow. Nevertheless, at the same time, it is a traumatic procedure because accessing through the thorax results in damage. Consequently, pneumothorax occurs. The severity of the surgical intervention is confirmed by mortality in the group of sham-operated animals (Shcherbak et al., 2012).

The objective of our study is to improve the 3VO model of GCI that was originally proposed by N.S. Shcherbak et al. (2012). We evaluated

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the effect of modified 3VO model of GCI in rats by examining the local cerebral blood flow dynamics in the visual cortex and neurological deficits. Besides, we carried out histological analysis of the hippocampus, which had also been done for the previous models of GCI (Liu et al., 2012; Pulsinelli and Brierley, 1979; Shcherbak et al., 2012).

2. Materials and methods

2.1. Animals

The study was carried out in accordance with the EU Directive 2010/63/EU concerning the protection of animals used for scientific purposes and approved by the Animal Care and Use Committee of Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NRMC (protocol No 22032012). Experiments were carried out on 65 adult male Wistar rats (250–300 g) obtained from the Department of Experimental Biological Models of E.D. Goldberg Institute of Pharmacology and Regenerative Medicine. Rats were housed in groups of five animals per cage (57 × 36 × 20 cm) in standard laboratory conditions (ambient temperature of 22 ± 2 °C, relative humidity of 60%, light/dark period 12/12 h a day) in cages with sawdust bedding and provided with standard rodent feed (PK-120-1, Ltd., Laboratorsnab, Russia), and *ad libitum* water access.

2.2. Experimental protocol

2.2.1. Designing the animal protocol

The animals were randomly divided into the following groups: I – sham-operated rats, II and III – 7-min GCI rats, IV – 15-min GCI rats, V – 7-min bilateral *aa. subclavian* system occlusion rats, VI – rats for evaluation of depth of GCI by means of Evans blue dye perfusion of the major cerebral vessels and VII – sham-operated rats to control staining of the brain with preserved patency of major cerebral vessels (Table 1). To study the reproducibility of the model when using anesthetics with different durations of action in groups II and III chloral hydrate anesthesia and propofol anesthesia have been used, respectively. Groups I–IV and VI–VII underwent the same operational procedure of initial preparation for the study, which consisted of pre-ligation of *a. subclavia sinistra, tr. brachiocephalicus* and *a. carotis communis sinistra.* In group V rats, the operational procedure ended with pre-ligation of *aa. subclavia.*

In groups I and II, local cerebral blood flow was registered continuously for at least 40 min.

In group II, ligatures were tightened after registering of local cerebral blood flow for 10 min, by means of which GCI was modelled. Then the ligatures were removed and brain reperfusion was performed. When the blood flow registration was complete, the rats were removed from the stereotaxic frame, and the wounds were washed with aseptic physiological solution and sutured. The rats were monitored closely until spontaneous respiration recovered before removing the endotracheal tube.

In rats from groups I, II, III, IV, V, the survival rate was registered after GCI or bilateral occlusion of *aa. subclavia*. On the first, third, and fifth days after the surgical procedure, a researcher who was unaware of the group identity of the rats performed a study of the neurological status.

On the fifth day after GCI, after the neurological status evaluation, subgoups of 5 rats each were taken from groups I and II and euthanized with CO_2. Then brain samples were taken for histological investigation.

In rats from groups VI and VII pre-ligation of *a. subclavia sinistra, tr. brachiocephalicus* and *a. carotis communis sinistra* was performed, heparine was administered intravenously, and the animals were euthanized with CO_2. In the rats from group VI the ligatures were tightened, while in the rats from group VII the ligatures were left untightened. The rats were perfused with Evans blue dye, and then the brain was removed, and the dorsal and ventral sides were photographed.

2.2.2. Anesthesia

The rats were anesthetized with chloral hydrate (Sigma-Aldrich Chemical Co.) at a dose of 450 mg/kg i.p. (groups I, II, IV–VII) or continuous propofol (AstraZeneca UK Ltd) infusion at a dose of 10 mg/kg/h i.v. (group III). Short-term anesthesia for implantation of an intravenous catheter was performed by ether (Kusbassorgchim, Russia) inhalation preparatory to full anesthesia.

2.2.3. Surgical procedure

The scheme of 3VO in rats is demonstrated in Fig. 1.

All surgical interventions were performed in aseptic conditions. The rats were anesthetized with chloral hydrate or propofol. For anesthesia with propofol, the rat was anesthetized with ether in a glass chamber until unconscious, and then a teflon i.v. cannula 26G was inserted into the femoral vein for continuous propofol infusion (10 mg/kg/h).

The rats were placed on a homeothermic blanket (Temperature Control Unit HB 101/2, Spain) in a supine position. The body temperature was maintained at 37 °C. Endotracheal intubation was performed through the oral cavity. The animals were breathing

Table 1
Distribution of animals into groups. Manipulation and studies carried out.

Groups	Blood vessel occlusion				Occlu-sion (min)	Re-circu-lation	Anesthesia		Investigations			
	a. subclavia		*a. carotis com.*				Chloral hydrate	Pro-pofol	Surviv-ability	Neurolo-gical deficit	Local cerebral blood flow	Histo-logical investi-gation
	dex.	*sin.*	*dex.*	*sin.*								
I Sham n = 7	–	–	–	–	–	–	+	–	+	+	+	+ (n = 5)
II n = 18	+	+	+	+	+ (7)	+	+	–	+	+	+	+ (n = 5)
III n = 19	+	+	+	+	+ (7)	+	–	+	+	+	+	–
IV n = 8	+	+	+	+	+ (15)	+	+	–	+	–	–	–
V n = 7	+	+	–	–	+ (7)	+	+	–	+	+	–	–
VI n = 3	+	+	+	+	+	–	+	–	–	–	–	–
VII Sham n = 3	–	–	–	–	–	–	+	–	–	–	–	–