DOI 10.1007/s10517-014-2721-8
Bulletin of Experimental Biology and Medicine, Vol. 158, No. 2, December, 2014 GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Evaluation of Survival and Neurological Deficit in Rats in the New Model of Global Transient Cerebral Ischemia

G. A. Chernysheva*, V. I. Smol'yakova*, A. N. Osipenko*, and M. B. Plotnikov*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 158, No. 8, pp. 159-161, August, 2014
Original article submitted August 17, 2013

We propose a modification to rat model of transient global cerebral ischemia with four-vessel occlusion avoiding pneumothorax and minimizing the consequences of surgery. Survival and neurological deficit in rats in this model was studied over 5 days.

Key Words: *global transient cerebral ischemia; brain; model; survival; neurological deficit*

Models of global transient cerebral ischemia (GTCI) are used in studies of the pathogenesis of cerebral ischemia and in the search for new drugs [2,4,10]. Rat GTCI model was developed by W.A. Pulsinelli and J.B. Brierley [6]. However, it has obvious shortcomings, such as two-stage simulation (electrocauterization of the vertebral arteries on experimental day 1 and occlusion of the carotid arteries on experimental day 2), partial vertebrobasilar insufficiency persisting after reocclusion of the carotid arteries, difficult access to the vertebral arteries, and risk of damage to the brain stem during electrocauterization of the vertebral arteries [6]. Subsequent modification of this model did not eliminate these significant drawbacks [7-9].

The new way of GTCI modeling in rats by reversible occlusion of the great arteries branching from the aortic arch and supplying the brain was proposed as an alternative method [1]. In anesthetized rats, the great vessels extending from the aortic arch were clipped via the chest approach. The advantages of this model are one-stage surgery and the absence of residual collateral blood flow. At the same time, it is quite traumatic due to damage to the pleural cavity leading to pneumothorax. Mortality in a group of sham-operated animals reflects the severity of the surgery [1].

The aim of this work was to improve GTCI model [1], namely to reduce surgical trauma, and to assess survival and neurological deficits in the animals after GTCI simulation by the proposed method.

MATERIALS AND METHODS

The study was performed on outbred Wistar male rats weighing 220-240 g obtained from the nursery of E.D. Goldberg Research Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences. Animal experiments were performed in accordance with the rules established by European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Surgery was performed under aseptic conditions. GTCI reperfusion model was reproduced by the method of [1] in our modification. The rats anesthetized with chloral hydrate (450 mg/kg intraperitoneally) were intubated without tracheal injury and then breathed spontaneously, because approach to blood vessels supplying the brain used by us excluded opening of the pleural cavity. Incision was made along the mid-ventral line of the neck; *a. carotis communis sin* was isolated and then ligated. On the ventral surface of the thorax at the level of the first intercostal space transverse skin incision was made extending above the first right costal cartilage, sternum, and first left

*Laboratory of Pharmacology of Blood Circulation, E. D. Goldberg Research Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences; **Laboratory of Phytochemistry and Experimental Pharmacology, National Research Tomsk State University; ***Siberian State Medical University, Ministry of Health of the Russian Federation, Tomsk, Russia. ***Address for correspondence:*** mbp2001@mail.ru. M. B. Plotnikov

costal cartilage. In the right intercostal space, intercostal muscles were cut between the first and second costal cartilages up to the sternum, the ribs were retracted, *tr. brachiocephalicus* was isolated proximally to the origin of *a. subclavia dextra*, *v. cava cranialis dextra* was gently retracted, and the isolated vessel was ligated with a small Cooper ligature needle. Then the intercostal muscles were cut in the left intercostal space between the first and second costal cartilages up to the sternum, the ribs were retracted, *a. subclavia sinistra* was isolated, *v. cava cranialis sinistra* was gently retracted, and isolated vessel was ligated using small Cooper ligature needle. After connecting a ventilator to the endotracheal tube in the open circuit, the ligated vessels were clamped for 7 min, after which the ligatures were removed, and wounds were sutured layer-by-layer. After the restoration of rhythmic spontaneous breathing, the animals were extubated. Sham-operated animals were subjected to the same manipulations except ligation of blood vessels.

Neurological deficit was assessed by McGraw Stroke Index scale [5] in our modification by the total score of the studied parameters. Higher nervous activity was assessed out on days 1, 3, and 5 after GTCI by the following parameters: spontaneous locomotor activity (normal, increased, decreased, or absent), gait disorders (stiffness, shakiness, slowness of movements, or disorientation), tail-flick response, withdrawal reflex, response to acoustic stimulation, tremors, convulsions, muscle tone in the trunk and legs (normal, increased, no tone), signs of ptosis (absence, one-sided, two-sided). Each parameter was scored from 0 to 2 (0 corresponded to normal; 1 and 2 corresponded to moderate and severe changes, respectively).

In each animal group, the proportion of animals with severe neurological disorders (total score ≥6), with moderate disorders (total score 3-5), and minor changes (≤2) was determined. The survival rate of the animals was recorded on days 1, 3, and 5 after GTCI.

Statistical processing was carried out using Statistica 6.0 software. The mean and standard error were calculated. The significance of differences ($p<0.05$) between the series was determined using Student's *t* test.

TABLE 1. Survival Rate in Animals after GTCI (%)

Group	Day after GTCI		
	1	3	5
Sham operation (*n*=10)	100	100	100
Ishemia (*n*=23)	69.6*	56.5*	47.8*

Note. Here and in Tables 2, 3: *$p<0.05$ in comparison with sham-operated animals.

TABLE 2. Mean Score of Neurological Deficit in Rats after GTCI

Group	Day after GTCI		
	1	3	5
Sham operation (*n*=10)	0.4±0.2	0	0
Ishemia (*n*=23)	9.3±1.7*	7.7±1.9*	4.4±0.9*

RESULTS

In the group of sham-operated animals, the surgery without ligation of the great vessels of the aortic arch caused no mortality (Table 1). On day 1 after surgery, the rats in this group showed only minor changes in spontaneous locomotor activity, which later disappeared. The average score of neurological deficit was 0.5±0.2 on day 1, no neurological disorders were detected at later terms of observation (Tables 2 and 3).

On day 1 after GTCI, 30.4% animals died in the experimental group, which roughly corresponds to survival described by the authors of the model [1]. Mortality increased to 43.5% on day 3 and to 52.2% on day 5 (Table 1).

TABLE 3. Neurological Status of Rats in the Studied Groups (% of Total Rat Number in the Group)

Severity of neurological deficit	Sham operation (*n*=10)			Ishemia (*n*=23)		
	day 1	day 3	day 5	day 1	day 3	day 5
Severe (>6)	0	–	–	68.8*	58.3*	30.0
Medium (3-5)	0	–	–	31.2	16.7	40.0
Mild (<2)	100	–	–	0*	25.0*	30.0

Note. "–" – no signs of neurological deficits were detected.

During the first 2-5 h after GTCI, experimental rats developed symptoms of CNS lesions, such as areflexia, seizures, spastic paralysis of the limbs, tonic tension in the muscles of the trunk and lateral position.

The mean score of neurological deficit on day 1 after GTCI modeling was 9.3±1.7 and later decreased (Table 2).

On day 1 after GTCI, more than $^{2}/_{3}$ of the experimental rats showed severe neurological disorders, and the rest have moderate degree of neurological deficit. In none animals, mild neurological deficit was observed. At later terms, the trend was observed for neurological status recovery (Table 3).

Thus, GTCI model induced by occlusion of the brachiocephalic trunk, the left subclavian artery and the left common carotid artery is associated with manifestations of severe and persistent neurological deficit. The access to the great vessels supplying the brain avoiding damage to the pleural cavity used in our modification makes the model less traumatic, reduces animal mortality, and, according to the results of survival in sham-operated rats minimizes the effects of surgery.

REFERENCES

1. N. S. Shcherbak, M. M. Galagudza, A. N. Kuzmenkov, *et al*., *Bull. Exp. Biol. Med*., **152**, No. 11, 656-658 (2012).
2. B. J. Alps and W. K. Hass, *Neurology*, **37**, No. 5, 809-814 (1987).
3. G. Bagetta, O. Chiappetta, D. Amantea, *et al*., *Neurosci. Lett*., **368**, No. 1, 87-91 (2004).
4. D. Lee, J. Park, J. Yoon, *et al*., *J. Ethnopharmacol*., **139**, No. 1, 6-11 (2012).
5. C. P. McGraw, *Arch. Neurol*., **34**, No. 6, 334-336 (1977).
6. W. A. Pulsinelli and J. B. Brierley, *Stroke*, **10**, No. 3, 267-272 (1979).
7. W. A. Pulsinelli and A. M. Buchan, *Stroke*, **19**, No. 7, 913-914 (1988).
8. M. Yamaguchi, J. W. Calvert, G. Kusaka, and J. H. Zhang, *Stroke*, **36**, No. 10, 2212-2214 (2005).
9. K. Sugio, N. Horigome, T. Sakaguchi, and M. Goto, *Stroke*, **19**, No. 7, 922 (1988).
10. F. Zhang, C. Li, R. Wang, *et al*., *Neuroscience*, **150**, No. 4, 938-49 (2007).