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Monitoring of Motor Disorders in 7-Day-Old Rats with Severe Hypoxic-Ischemic Injury of the Brain

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Seven-day old rats were subjected to unilateral ligation of the common carotid artery followed by 2-h exposure to a gas mixture consisting of 8% oxygen and 92% nitrogen. Locomotor function of rats was monitored weekly. Functional deficit in these animals persisted for at least 3 months. The exercise tests of rotarod, hanging, and narrowing track were most informative. Our results can be used in preclinical studies of new drugs for the therapy of perinatal brain injury.

Key Words: *perinatal hypoxic-ischemic brain injury; functional exercise tests*

Published data show that one-third of patients with severe perinatal hypoxic-ischemic brain injury (PHIBI) have persistent motor disorders (*e.g.*, infantile cerebral palsy) [3]. Much attention is paid to new therapeutic methods for these disturbances (cellular and genetic therapy, hypothermia, directed transport of bioactive substances, *etc*.). Basic researches with new pharmaceutical products cannot be performed on newborns. Therefore, the possibility of using these agents in medical practice is determined by the results of preclinical studies on animals with experimental PHIBI [1,5,8].

The Rice–Vannucci model (unilateral ligation of the common carotid artery and subsequent exposure of rat pups to a gas mixture with low oxygen content) adequately reflects clinical manifestations of severe PHIBI [5,7]. The major pathogenetic stages of acute PHIBI and efficacy of neonatal emergency drugs were estimated on this model [6]. However, little is known about long-term consequences of PHIBI. The animal models of PHIBI should be adapted to the search for new therapeutic approaches to the chronic stage of this disease. For example, it is necessary to increase the duration of studies (up to more than 8 weeks) [4] and to use the functional exercise tests. These tests verify the efficacy of therapeutic methods at the level of the whole body. Hence, the results of these experiments can be extrapolated to clinical practice [1]. It is important to select some test for the functions that are characterized by the lowest degree of spontaneous recovery.

Here we performed 12-week monitoring of motor disorders in rats with PHIBI (Rice–Vannucci model) and selected the most reliable tests for motor deficit.

MATERIALS AND METHODS

Experiments were performed on rat pups (n=81) from outbred albino males and females aging 4-5 months. The litters were randomly divided into the control and treatment groups.

PHIBI in 7-day-old rats of the treatment group was induced by the method of Rice–Vannucci [7]. The left common carotid artery was cut between two liga-

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tures (silk 3-0) under halothane inhalation anesthesia and sterile conditions. The animals were maintained in a warm chamber (35-36°C) for 120 min. Then these rats were put in a 2-liter chamber. A moistened and warm gas mixture (36°C) containing 8% oxygen and 92% nitrogen was delivered through the chamber (rate 6 liter/min). Stereotyped head-shaking and generalized clonic seizures were observed in 50% rat pups after 20-40 min. This exposure caused death of 5 rat pups. Control animals were subjected to all manipulations except for cutting the carotid artery and exposure to atmosphere with low oxygen content. Control animals did not die under these conditions. In the follow-up period, rat pups were returned to individual maternal cages. The daylight period was 12 h. The animals had free access to food and water.

Rat pups of the control (*n*=42) and treatment groups (*n*=34) were weighted weekly for 3 months. The degree of functional deficit was estimated at the age of 3-4 months and older (age of rat pups after birth). The exercise tests of hanging, rotarod, and narrowing track were performed. Plantographic indexes were evaluated. Motor asymmetry was detected from the angular test (K-test).

"Hanging". Animal's forelimbs were brought to a rope (diameter 2 mm) positioned at a height of 80 cm from the floor level. The rats clutched at this rope with forelimbs and hung. The latency of falling off the rope (seconds) was recorded.

"Rotarod". Rat pups were placed on a rotating cylinder (diameter 70 mm, rotation rate 7 rpm; Neirobotiks). In repeated sessions of the rotarod test, the animals were trained to retain on this cylinder for at least 1 min. After 15-20 min, the time of staying at a rotation rate of 21 rpm (seconds) was recorded.

"Narrowing track". The rats moved voluntarily over a narrowing track. The length of this track was 165 cm. The initial and final width of the track was 6 and 2 cm, respectively. The track was positioned at a height of 70 cm from the floor level. The track came to an end in the home cage. The number of hindlimb sliding off the track (errors) was recorded separately for the left and right side. Asymmetry was evaluated from the difference between the numbers of leftward and rightward errors.

"Swimming". We recorded the time of crossing the pool (length 120 cm, width 10 cm).

"Angular test". The rats moved voluntarily into an angle (30°), which was formed by 2 walls (height 30 cm). When the animals reached this angle, they turned in a leftward or rightward direction. We recorded the number of leftward and rightward rotations. The test was performed 5 times. The mean value of asymmetry was calculated as the difference between the numbers of rightward and leftward rotations.

"K-test" [2]. The animals received an intraperitoneal injection of ketamine (50 mg/kg) and were placed in an automated rotameter. A rotation counter was turned on after 2 min. The rate of rotations (rpm) in various directions was recorded over 30 min. The mean value of asymmetry was calculated as the difference between the rates of leftward and rightward rotations.

"Plantography". The rats moved across a tunnel (width 7 cm, length 1.3 m). A paper tape was placed on the floor of this tunnel. The limbs of animals were dipped in ink to obtain the prints. We measured the width of prints (distance between the endpoints of finger I and finger IV on all limbs, mm) and length of steps (distance between the midpoints of pads, mm). The mean of 3 measurements was calculated.

The results were analyzed by nonparametric Mann–Whitney test (for absolute values), χ^2 test and exact Fisher test (for relative values).

RESULTS

Control rats and treated animals did not die over 12 weeks of the study. Body weight gain in rat pups with PHIBI did not differ from the control.

The exercise tests, motor asymmetry tests, and plantography showed that induction of PHIBI (Rice–Vannucci model) [7] is followed by motor deficit, which persist for at least 12 weeks of postnatal development (*i.e.*, persisted in adult animals). The significance of tests for motor disorders was different (Table 1). The mean values of the angular test and plantography in rat pups with PHIBI significantly differed from the control only over the 1st month of life. No significant differences were found in the induced motor asymmetry (K-test) and swimming speed of control animals and treated specimens. The rotarod test, hanging test, and narrowing track test demonstrated the existence of severe dysfunction in the delayed period (by the 5th-12th week of monitoring). Statistical analysis was performed to compare the percentage of animals with certain levels of spontaneous recovery of impaired functions (Table 2). High informativeness was shown not only for the rotarod, hanging, and narrowing track tests, but also for the K-test and angular test. Calculating the mean values revealed only a tendency to the increase in motor asymmetry (Tables 1 and 2).

The relative values were compared for PHIBI rats and control animals (100%). Functional recovery was not found in the hanging test and narrowing track test. Spontaneous recovery was revealed in the rotarod test on the 11th and 12th weeks of monitoring (Fig. 1). The relative value of the angular test varied during the experiment. Temporal improvement of functions was shown to alternate with dysfunction. A similar

TABLE 1. Mean Values of Functional Sate of PHIBI Rats ($M \pm m$)

Parameter, group		Age, weeks									
		3	4	5	6	7	8	9	10	11	12
Rotarod, sec	control		69±9	72±8	115±21	110±20	92±17	78±18	68±6	41±5	61±3
	treatment		31±8*	40±5*	41±6*	46±6*	38±4*	33±8	27±18	40±13	57±32
Hanging, sec	control	163±26	212±27	129±14	121±18	113±16	101±17	134±39	135±24	119±32	150±30
	treatment	89±19*	101±19*	170±19	134±15	133±23	90±16	46±8*	48±12*	19±3*	18±2*
Narrowing track, total number of errors	control			0.9±0.2	1.4±0.3	1.4±0.2	1.1±0.2	0.3±0.2	0.5±0.2	0.3±0.2	0.3±0.2
	treatment			1.8±0.3*	2.1±0.3	1.7±0.2	1.5±0.2	0.8±0.3	1.0±0.4	0.6±0.3	1.2±0.5
Narrowing track, asymmetry of errors	control			0.1±0.1	0.1±0.2	0.1±0.3	0.3±0.2	0.3±0.2	0.0±0.3	0.3±0.2	0.3±0.2
	treatment			0.6±0.3*	0.5±0.4	1.1±0.3*	1.0±0.3	0.8±0.3	1.0±0.4	0.6±0.2	1.1±0.4
Swimming, sec	control				32.8±4.9	7.3±0.6	7.0±0.5	6.9±0.4	6.7±0.5	8.0±0.6	9.2±1.4
	treatment				27.6±5.2	9.1±0.6	8.2±0.6	8.1±0.5	7.9±0.5	10.2±0.4	9.8±1.3
K-test, rpm	control		0.8±2.1	0.8±1.1	0.3±2.2	0.0±2.3	0.7±1.2	0.6±1.5	0.7±0.9	0.9±0.7	0.6±2.0
	treatment		4.2±3.3	1.7±0.9	4.1±2.5	2.2±2.4	2.0±4.9	2.6±2.6	1.6±3.7	1.6±1.1	2.5±0.6
Angular test, asymmetry of rotations	control	0.0±0.3	0.6±0.3	0.4±0.7	0.2±0.7	0.1±0.5	0.0±0.4	1.5±0.6	1.5±0.3	1.0±0.7	0.6±0.7
	treatment	1.9±0.5*	1.7±0.8*	0.0±1.1	0.0±0.9	0.8±0.6	1.4±0.4	1.7±0.6	0.2±1.1	2.5±0.3	2.0±1.4
Step length, mm	control		86.7±2.6		113.7±3.2		111.4±3.5		108.5±3.8		111.1±4.3
	treatment		93.9±2.3*		107.9±4.7		109.2±3.0		103.9±5.4		103.8±8.1
Print width of the left forelimb, mm	control		14.2±0.3		16.2±0.3		16.4±0.4		16.8±0.6		18.1±0.9
	treatment		13.3±0.2*		15.8±0.5		16.3±0.4		16.1±0.5		16.8±0.6

Note. *$p<0.05$ compared to the control (Mann–Whitney test).

TABLE 2. Statistical Analysis of Exercise Tests in PHIBI Rats

Parameter	Age, weeks									
	3	4	5	6	7	8	9	10	11	12
Mean time of stay in the rotarod test, sec		⇓	⇓	⇓	⇓	⇓	↓	↓	Nd	Nd
Percent of rats staying on a rotarod >60 sec, %		⇓	⇓	⇓	⇓	⇓	⇓	⇓	↓	↓
Mean time in the hanging test, sec	⇓	⇓	↑	↑	↑	↓	⇓	⇓	⇓	⇓
Percent of rats hanging on the rope >120 sec, %	⇓	⇓	↑	↑	↑	↓	↓	↓	⇓	⇓
Narrowing track, mean total number of errors			⇑	↑	↑	↑	↑	↑	↑	↑
Narrowing track, percent of rats with contralateral asymmetry of errors			⇑	⇑	⇑	⇑	↑	↑	↑	↑
Mean speed of rotation (K-test), rpm		↑	↑	↑	↑	↑	↑	↑	↑	↑
Ratio of rats with rotation speed ≥2 rpm (K-test), %		⇑	⇑	⇑	⇑	⇑	⇑	⇑	⇑	⇑
Angular test, mean difference between the numbers of rightward and leftward rotations	⇑	⇑	↑	↑	Nd	↓	Nd	↓	↓	↓
Angular test, percent of rats with ipsilateral asymmetry of rotations	⇑	⇑	⇑	↑	↑	⇓	Nd	↓	⇓	⇓

Note. Up arrows and down arrows: increase and decrease in the parameter, respectively. Double arrow: $p<0.05$; arrow: $0.1>p>0.05$; Nd: no differences.

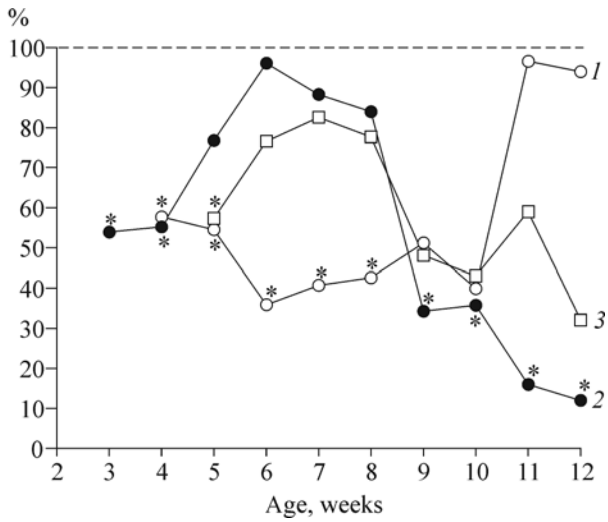

Fig. 1. Recovery of motor functions in PHIBI rats. Rotarod (*1*); hanging (*2*); narrowing track (*3*). *p<0.05 compared to the control (100%).

study with the K-test was impossible due to significant variability of the results. The degree of motor deficit in randomly selected animals of the control (n=5) and treatment groups (n=3) was evaluated in the rotarod test. This index in treated rats was much lower than in controls (30.0±6.5 and 62.7±23.6 sec, respectively).

Our results indicate that the exercise tests for tolerance, coordination, and working capacity (hanging, narrowing track, and rotarod) should be used for the detection of persistent motor deficit in rat pups with PHIBI. The studies should be performed for at least 3 months of postnatal development (*i.e.*, in adult animals). Objective evaluation of general functions can be achieved by comparing not only the mean values, but also the percentage of control specimens and treated animals with a certain level of spontaneous recovery of impaired functions. This approach allows us to use the tests for induced and spontaneous motor asymmetry (K-test and angular test, respectively). The proposed method can be used in preclinical evaluation of the efficacy of new drugs for perinatal CNS injury.

REFERENCES

1. S. V. Lebedev, A. V. Karasev, S. O. Rogatkin, *et al.*, *Vestn. Ros. Akad. Med. Nauk*, No. 2, 21-26 (2009).
2. S. V. Lebedev, S. V. Petrov, D. V. Blinov, *et al.*, *Byull. Eksp. Biol. Med.*, **135**, No. 3, 500-505 (2003).
3. F. Allemand, F. Reale, M. Sposato, and A. Allemand, *Ital. J. Pediatr.*, **35**, No. 1, 14 (2009).
4. W. Balduini, V. De Angelis, E. Mazzoni, and M. Cimino, *Brain Res.*, **859**, No. 2, 318-325 (2000).
5. M. V. Johnston, D. M. Ferriero, S. J. Vannucci, and H. Hagberg, *J. Child Neurol.*, **20**, No. 12, 984-987 (2005).
6. F. Northington, *ILAR J.*, **47**, No. 1, 32-38 (2006).
7. J. E. Rice, R. C. Vannucci, and J. B. Brierley, *Ann. Neurol.*, **9**, No. 2, 131-141 (1981).
8. J. Y. Yager and S. Ashwal, *Pediatr. Neurol.*, **40**, No. 3, 156-167 (2009).