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Original Paper

Somatosensory Evoked Potentials in Cerebral Infarction Model Rats Induced by Microsphere Injection to Cerebral Artery

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Abstract

Microspheres, monodisperse polystyrene beads, are used for the study of cerebral infarction by injection into the cerebral artery. Cerebral infarction model rats were made by injecting 2,600 microspheres into the right internal carotid artery in this study. Motor ability was assessed by the rotarod test in which cling duration on the accelerating rotating rod was measured. Significant decrease in the motor ability was recognized on 3 and 15 days after the embolization compared with before surgery ($p<0.05$). However, sham-operated rats did not show significant change of motor ability. Each rat was reanaesthetized 15 days after surgery, and somatosensory evoked potentials (SEPs) induced by stimulation of the sciatic nerve of the contralateral hind limb were averaged. Latencies of N_1 and N_2 waves in SEPs were compared between sham-operated rats and embolized rats 15 days after surgery. No significant differences in N_1 and N_2 latencies were recognized between the two groups. These results suggest that motor ability was impaired at chronic periods of the embolization, while SEPs recovered, although latencies of N_1 and N_2 in SEPs were reported to increase at acute periods in embolized rats.

Introduction

Vascular dementia is the most common form of dementia in Japan and usually affects elderly people. With a rapidly increasing aged population, the care of demented elderly has become an important social problem. The most common type of vascular dementia is multi-infarct dementia, which is caused by a series of small strokes that often go unnoticed and cause damage to the cortex of the brain, the area associated with learning, memory, and language. In rat models, transient four-vessel occlusion [1–2] and bilateral carotid artery occlusion [3] demonstrated gradual development of multiple infarction and/or white matter changes in association with memory impairment. Unfortunately, none of these models provide the multiple small subcortical infarctions as like characteristics of multi infarct dementia patients. Recently microspheres have been used to produce a permanent occlusion of microvessels. Microspheres are monodisperse polystyrene beads supplied in sizes from 50nm to 90μm. The microsphere-induced cerebral embolism model is a multifocal model of permanent occlusion [4]. The size and the dose of the injected microspheres can be used to regulate the extent and severity of the resulting lesions [5]. In a previous study, microsphere embolism induced a marked decrease in blood flows and a pronounced disturbance of energy metabolism

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in the brain region [6, 7]. Since multiple small infarctions are a common cause of vascular dementia, this embolic model resembles clinical vascular dementia closely. In this study, we produced microsphere-induced cerebral embolism model rats and assessed motor impairment with the use of the accelerating rotarod.

Monitoring of somatosensory evoked potentials (SEPs) could be of benefit in guiding cerebroprotective therapy [8]. SEPs are valuable in experimental stroke studies in various animal species [9–12], also in rodent models [13–15]. In a cat model after occlusion of the right middle cerebral artery, all cats using the study demonstrated slowing of the interpeak latency on the right hemisphere recordings as compared to presurgery at 1 hour post occlusion [12]. In a study using rats, abnormalities in the SEP are known to occur in association with lesions in the hemisphere at an acute period [13]. Significant delay and incomplete recovery of each latency was observed in the 2 hours middle cerebral artery occlusion +2 hours reperfusion rats at the end of observation period of 120 min as compared to preocclusion group [14]. In chronic period after ischemia, one study indicated that the SEP was absent 10 days after permanent cerebral artery occlusion in the majority of rats and the latency was unaffected in the remainder [15]. Thus, the latency of SEP in the chronic period has not been well investigated. In this study, we have examined the effect of microsphere-induced cerebral embolism on SEPs in the rat at a chronic period.

Materials and Methods

1. Animals

Male Sprague-Dawley rats (Clea Japan Inc., Meguro, Japan), weighing 390.9 ± 28.4g, were maintained with a 12-h light/12-h dark cycle throughout the experiment (n=13). The animals had free access to food and water. All experiments were conducted according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science (Physiological Society of Japan, 2003). The study protocols were approved by the Institutional Animal Care and Use Committee in Kawasaki University of Medical Welfare (No. 08–019).

2. Microsphere Embolism

Microsphere (45 μm in diameter, Polybead Polystyrene Microspheres, Polyscience) was used for induction of cerebral embolism performed by the method described previously [5, 6] with some modification. Rats were anesthetized with intravenous 0.7g/kg urethane and 0.06g/kg α-chloralose. After exposure of the right common carotid artery, the right external carotid and pterygopalatine arteries were temporarily occluded with clamps (micro serrefine18055–01, FST). The dextran solution (saviosol, Otsuka Pharmaceutical) containing approximately 2,600 microspheres was injected through the right common carotid artery at 0.15ml/30sec using an electric injector (SP120p, KD SCIENTIFIC inc), according to methods in a previous paper [16]. After the temporal occlusion of the arteries, the blood flow was reestablished within 3 min. Sham operated rats were injected with the same volume of dextran solution without microspheres.

3. Assessment of Functional Outcome

Motor impairment due to microsphere injection was assessed with the use of the accelerating rotarod (MK–630A, Muromachi co). Rotarod tests were carried out pre-operation, 3 and 15 days after surgery. The duration that rats remained on the accelerating rotating rod was measured. The rotating velocity was gradually increased from 4 m/min to 40 m/min within 5 minutes. A trial was terminated when the rat fell down from the rotarod. The average time of five trials was used for data analysis.

4. Electrophysiological Recordings of SEPs

Fifteen days after embolization, each rat was anaesthetized with intravenous 0.7g/kg urethane and 0.06g/kg α-chloralose. Supplements were administered if necessary in order to maintain areflexia until the end of the surgery. Body temperature was maintained at 37℃ with a heating pad. Animals were secured in a stereotaxic head folder. The skull was exposed and sufficient bone was removed with a drill to produce bilateral windows extending from bregma to 3 mm posterior to bregma, and 4mm laterally from sagittal suture. A silver ball recording electrode (diameter: 50μm) with a shielded cable was held in a micromanipulator and lowered onto the exposed dura overlying the somatosensory cortex of one hemisphere. A reference electrode was inserted into neck muscle. A ground lead was attached to an ear bar. The sciatic nerve of the contralateral hind limb was stimulated with a bipolar electrode. Current pulses (0.3ms, 1.7Hz) were delivered from the electronic stimulator (SEN–3301; Nihon Koden) with an intensity ranging around 300 μA. The intensity of stimulation was fixed at a level that evoked a slight movement of the limb. The raw signal was digitized at 20 kHz into a personal computer using an AD converter (Powerlab/4S). Responses were then averaged 128 times with Scope software (AD instruments). Latencies were defined as the time from the onset of the stimulus to the peak of each wave of the somatosensory evoked potential. Recordings were made from both hemispheres and the potentials were always evoked by stimulation of the contralateral sciatic nerve.

5. Statistical Analysis

Statistical analysis was performed using statistical software (SPSS for Windows). The data are presented as mean ± S.D. Student's t-test, the paired t-test and Dunnett's test were used. The level of significance was set at $P<0.05$.

Results

1. Evaluation of Neurological Deficits

Two days after surgery, the behaviors of microsphere-injected (n=8) and the sham-operated rats (n=5) were evaluated on the basis of paucity of movement, truncal curvature, and forced circling during locomotion, which were considered to be typical symptoms of stroke in rats [17, 18]. In microsphere-injected rats, these neurological deficits were marked within 3 days after embolization, and then gradually recovered. Two rats which did not show apparent neurological deficits were excluded from this study. Sham-operated rats showed no obvious neurological deficits 3 days after surgery.

2. Comparison of Motor Function between Embolized Rats and Sham-operated Rats

To assess motor impairment, we used the rotarod test (see Methods). The duration that a rat remained on the accelerating rotating rod was measured pre-operation, 3 and 15 days after embolization and sham-operation. The value was expressed in the ratio to motor ability at pre-operation. The ratio between the value at pre-operation and that of value 3 and 15 days after surgery was expressed as motor ability. In embolized rats, a significant decrease in relative value of motor ability was observed on 3 and 15 days after embolization compared with pre-operation ($p<0.05$, Fig. 1). In sham-operated rats, no significant difference was recognized in relative value of motor ability. Compared with sham-operated rats, the relative value of motor ability of embolized rats significantly decreased on 3 days after surgery ($p<0.05$).

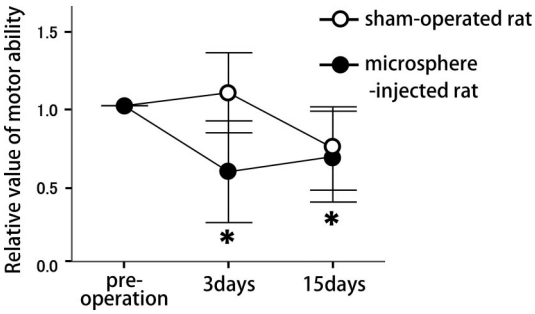

Fig. 1 Relative value of motor ability evaluated using the rotarod test. Values are indicated by means ± S.D. *p<0.05, compared with pre-operation, 3 and 15 days after surgery.

3. Somatosensory Evoked Potentials Induced by Sciatic Nerve Stimulation

An example is shown in Fig. 2. A computer-averaged SEP recorded from the bilateral hemisphere of an anaesthetized and embolized rat is shown. The raw signal was digitized at 20 kHz into a personal computer. Responses were then averaged 128 times with Scope software. The stimulation of the sciatic nerve evoked electrical activity with prominent first-negative (N_1), first-positive (P_1) and second-negative (N_2) components; all SEPs had these components. A negative wave was expressed as an upward deflection. Table 1 shows the mean ± S.D of the latency of N_1 and N_2 of ipsilateral and contralateral hemispheres in sham-operated rats and embolized rats. In sham-operated rats, on the ipsilateral hemisphere the latencies of N_1 and N_2 occurred at 13.34 ± 0.71 msec and 20.62 ± 0.97 msec, and on the contralateral hemisphere at 13.34 ± 1.29 msec and 20.92 ± 1.32 msec, respectively. In embolized rats, on the ipsilateral hemisphere the latencies of N_1 and N_2 occurred at 13.44 ± 1.02 msec and 20.96 ± 1.98 msec, and on the contralateral hemisphere at 13.48 ± 0.43 msec and 20.00 ± 0.97 msec, respectively. There were no significant differences in the latency of the SEP between ipsilateral and contralateral hemispheres in sham-operated and also embolized rats. Moreover, there were no significant differences in the latencies of the SEPs on both hemispheres between sham-operated rats and embolized rats.

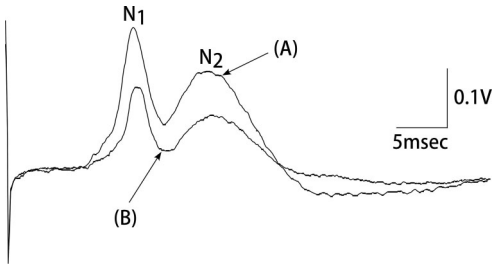

Fig. 2 An example of computer-averaged SEPs recorded from an embolized and anaesthetized rat. The SEP (A) was recorded from the contralateral hemisphere to the microsphere injected artery, and SEP (B) was recorded from the ipsilateral side.

Table 1 Latencies of N_1 and N_2 waves recorded from both hemispheres.

	N_1 wave latency (ms)		N_2 wave latency (ms)	
	ipsilateral	contralateral	ipsilateral	contralateral
sham operated (n=6)	13.34±0.71	13.34±1.29	20.62±0.97	20.92±1.32
microsphere-injected (n=5)	13.44±1.02	13.48±0.43	20.96±1.98	20.00±0.97

Values are indicated by means ± S.D.
Statistical differences were not observed in each value.

Discussion

Motor Function of Embolized Rats

In this study, some neurological deficits were recognized in embolized rats by microspheres injected to the internal carotid artery, while no obvious neurological deficits were observed in sham-operated rats 3 days after surgery. Additionally, motor ability evaluated using a rotarod significantly decreased in microsphere injected rats 3 and 15 days after surgery. In contrast, motor ability of sham-operated rats did not show significant changes. It is known that the rotarod task required the rat to maintain its equilibrium sense and/or coordinated movement ability [19], and rotarod testing was extensively employed to evaluate motor ability of infarcted animals [20]. Thus, equilibrium sense or coordinated movement ability of embolized rats seemed to be damaged until 15 days after surgery. Takeo et al. [21] also reported that the behavior of microsphere-injected rats gradually improved with time after the operation, and most rats still exhibited neurological deficits on 3 days after surgery. However, there were no significant differences in behavioral neurological deficits between microsphere-injected rats and sham-operated rats 6 days after surgery in their study. Moreover, swimming speed in the water maze test of microsphere-injected rats was reportedly similar to that of sham-operated rats on 12–14 days after surgery [22]. It is reported that the rotarod test has been used not only to evaluate motor function but also learning of movements [23, 24]. This may be the reason why the rotarod task was impaired in microsphere injected rats even 15 days after surgery.

On the other hand, it is reported that a microsphere embolism impaired forebrain cholinergic neurotransmission, and obstructed cognitive function [25]. Kiyota et al. [26] suggested that microsphere-injected rats recovered from impaired acquisition of passive avoidance response in a time-dependent way, but that the long-term memory indicating latencies of passive avoidance response were impaired in 14 days after surgery. Moreover, estimation of learning and memory function using the Morris water maze test was significantly depressed in microsphere-injected rats compared with sham-operated ones 14 days after the surgery [27]. Hence, integrative motor ability using the rotarod test might be impaired by cognitive function disorder in microsphere-injected rats in this study even 15 days after the surgery.

Latencies of Somatosensory Evoked Potentials Evoked by Sciatic Nerve Stimulation

The waveforms of the SEPs in animals are known to be generated by post synaptic potentials from specific structures in the central nervous system [28]. According to Koyanagi and Tator, the N_1 wave of SEP likely reflects activities from deep structures such as the thalamus, and is widely distributed over the cerebral cortex [29]. The N_2 wave likely reflects activities from superficial structures of cerebral cortex, and is sharply localized to a small area. The rat primary somatosensory cortex is anatomically and physiologically defined as distinct regions. Therefore the N_2 wave of SEP might be corresponding to activity from sensory cortex of hindlimb area.

Previous investigations with focal ischemia demonstrated that amplitude of the cortical SEP is sensitive to oligemia, and that ischemia tolerance of the SEP amplitude is higher in fiber tracts than neurons in the cortex [9, 10]. However, latency of SEP could be due mainly to white matter function. Thus, white matter blood flow is reportedly correlated to the latency of the SEP in global ischemia [30].

Many investigators indicated that cerebral blood flow was reduced after middle cerebral artery occlusion (MCAO) [10, 30–32]. In cats, the interval of N_1 and N_2 peak on the right hemisphere prolonged after right MCAO at 1 hour post occlusion. In rats, a significant delay of the latency of N_1 was observed by 2 hours MCAO [14]. In contrast, there were no significant differences in latency of N_1 and N_2 peak between microsphere-injected and sham-operated groups. These differences may be due to periods for recovery since changes of latencies were observed in the acute study described above, while the SEP was recorded

in a chronic period in our study. In fact, the cerebral blood flow of microsphere-induced rats significantly declined at 48 hours after surgery using the MRI study [4], while the high intensity areas on T2-weighted images induced by microsphere embolism were markedly diminished on 8 days after the surgery [33]. Thus, no significant latency changes of the SEP were recognized in our chronic study. Taken together, the latency of SEP might be recovered because only oligosynapse mediated in the central nervous system. However, higher functions such as cognition, learning and memory etc. which are related to polysynaptic brain function could be damaged by microsphere embolism in the chronic periods.

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