Full title:

Changes in the lactate threshold during treadmill exercise after microsphere-induced infarction in rats

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Short title: Lactate threshold changes after stroke

Abstract

Objectives The aim of this study was to clarify changes in the lactate threshold (LT) in the acute period after cerebral infarction.

Methods Cerebral infarction was induced by the injection of microspheres (MS) into the right internal carotid artery. To estimate the degree of neurological deficit caused by surgery, the behaviors of all rats were evaluated in terms of typical symptoms of stroke in rats. A rotarod test was used to evaluate equilibrium function. Rats were forced to perform stepwise treadmill exercises and serial changes in the blood lactate concentration were measured for determination of the LT.

Results The average treadmill speed at the LT and rotarod performance in MS rats was significantly lower than those in sham-operated rats on the 2^{nd} days after surgery. However, while neurological deficits disappeared on the 7^{th} days after surgery in MS rats, the LT and rotarod performance were significantly lower than those in sham-operated rats.

Conclusion These results suggest that the decrease in the LT in the acute period following cerebral infarction may be induced by impairment of the equilibrium function. Other possibilities are also discussed.

Key words: lactate threshold, microsphere, rotarod, treadmill, cerebral infarction

INTRODUCTION

While the concentration of blood lactate is known to remain low during light exercise, lactate begins to accumulate when the exercise intensity is gradually increased. The inflection point at which lactate accumulation begins is referred to as the lactate threshold (LT) [1]. The LT is the level of exertion at which the body must switch from aerobic metabolism to anaerobic metabolism. Exercise above the LT is associated with an increase in metabolic, respiratory and perceptual stress [2, 3]. Furthermore, exercise above the LT is associated with more rapid fatigue, either through the effects of metabolic acidosis on contractile function or through an accelerated depletion of muscle glycogen [4, 5]. On the other hand, exercise below the LT level is safe and can be sustained without excessive exertion [6]. Therefore, the LT is an optimal index for safe and effective exercise.

Stroke patients show poor physical fitness and require exercise to improve their physical fitness without harming their physical condition. Thus, it is assumed that it is important to measure the LT to ensure that stroke patients exercise safely. It is known that the LT of stroke patients is low in the chronic period [7]. Patients in the acute period after stroke are more vulnerable to any deleterious effects of excessive exercise than those in the chronic period. Therefore, it may be more important to determine the LT in the acute period after stroke than in the chronic period. However, the changes in the LT in the acute period after stroke are unclear.

It has been reported that prolonged inactivity in the chronic period following stroke leads to poor cardiorespiratory fitness [8], poor balance, and muscle weakness [9]. These decreases in cardiorespiratory function and muscle strength lower the LT [10]. After stroke, most patients show impaired equilibrium function and their gait patterns are unstable [11]. Energy expenditure in stroke patients was higher than that in healthy subjects during walking at a slow speed [12]. This relative increase in energy expenditure during walking at the same speed may accelerate the switching from aerobic metabolism to anaerobic metabolism. Thus, the LT of stroke patients in the acute period may be low, similar to that in the chronic period.

In this study, rats that had experienced cerebral infarction were forced to perform treadmill exercises and serial changes in the blood lactate concentration were investigated. The purpose of this study was to clarify changes in LT in the acute period after cerebral infarction induced by the arterial injection of microspheres, which is known to produce the permanent occlusion of cerebral microvessels [13, 14].

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 318.6 ± 32.0 g (n=29), were maintained under a 12-h light/12-h dark cycle throughout the experiment. 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, 2009). The protocols of this study were approved by the Institutional Animal Care and Use Committee of Kawasaki University of Medical Welfare (No. 08-019).

Investigation of lactate threshold (LT) and equilibrium function: A multistage treadmill (MK-680, Muromachi, Japan) was used to raise blood lactate levels in the training period in 17 rats. A session of training, in which a rat was forced to run at 15 m/min at a treadmill inclination of 10° for 10 min, was conducted 5 times over 3 days. After the training period, the LT of each rat was determined as follows. A drop of blood was sampled from the vein of the animal's tail according to methods reported by Fluttert et al. [15], and the lactate concentration was measured using a portable lactate analyzer (Lactate Pro, Arkray, Japan). The rat then ran at an initial speed of 10 m/min at a treadmill inclination of 10° for 3 min. The rat performed several exercise sessions with an increase in the treadmill velocity. At each session, the speed was increased by 5 m/min compared to the previous session until it reached 35 m/min. Each exercise load lasted 3 min and was followed by 1 min of rest during which blood samples were taken. The LT was determined from individual plots of blood lactate vs. treadmill speed using software (MEQNET LT Manager, Arkray, Japan). The software determined the two-line combination that minimizes the total sum of squares for fitting all data. The LT was

considered to be at the intersection of the two lines [16].A rotarod test using a rotarod apparatus (MK-630A, Muromachi, Japan) was used to evaluate equilibrium function using 12 other rats. Rats were trained to run on an accelerating rotating rod under a gradual increase in speed from 2 to 30 m/min for 5 min, 5 times over 3 days. After the training period, the amount of time that the rats remained on the accelerating rotating rod was measured. The average duration in 3 sessions was used for data analysis.

Cerebral infarction using microspheres: All rats were anesthetized with intravenous 0.7g/kg urethane and 0.06g/kg α-chloralose. A midline incision was made on the ventral side of the neck. After the right common carotid artery was exposed, the right external carotid and pterygopalatine arteries were temporarily occluded with small clamps. Microspheres (45 μm in diameter, Polybead Polystyrene Microspheres, Polysciences, USA) were used to induce cerebral infarction according to a method described previously [13, 14]. A dextran solution (Saviosol, Otsuka Pharmaceutical, Japan) containing approximately 500 microspheres was injected through the right common carotid artery at 0.2ml/30sec using an electric injector (KDS120, KD Scientific, USA) in 17 rats (MS rats). The same volume of dextran solution without microspheres was injected in the other 12 rats (sham rats). The blood flow was immediately reestablished after injection. The surgical incision was then closed with sutures and rats were kept on a heated surgical pad until they had recovered from anesthesia to maintain body temperature. On the 1^{st}, 2^{nd} and 7^{th} days after surgery, neurological deficits were scored according to previously reported criteria [17, 18]. Evaluations of the LT and equilibrium function were conducted at pre-surgery and on days 2 and 7 after surgery.

Statistical Analysis: Statistical differences were evaluated using Friedman's test, Steel-Dwass test, Mann-Whitney U test, two-way repeated measures analysis of variance, Dunnett’s test and unpaired t-test. P-values less than 0.05 were considered significant. P-values less than 0.1 and more than 0.05 were considered to show a tendency.

RESULTS

Neurological deficit scores: To estimate the degree of neurological deficit caused by surgery, the behaviors of MS (n=17) and sham rats (n=12) were evaluated in terms of paucity of movement, truncal curvature, and forced circling during locomotion, which were considered to be typical symptoms of stroke in rats [17, 18]. Each item was given a score from 0 to 3 (0, little or none; 1, moderate; 2, severe; 3, very severe). The sum of these scores was used as a marker of neurological deficits. Fifteen MS rats showed total scores of 5-9 points on the day after surgery. The other two MS rats showed scores of less than 5 points, and were excluded from this study. Neurological deficit scores on the 2^{nd} and 7^{th} days after surgery were significantly lower than those on the day after surgery in MS rats (Fig. 1A, $p<0.01$, Friedman's test and Steel-Dwass test). In sham rats, a slight neurological deficit was seen on the day after surgery, but no deficit was observed on the 2^{nd} and 7^{th} days after surgery. Significant differences in neurological deficit scores were recognized between MS and sham rats on the 1^{st} and 2^{nd} days after surgery (Fig. 1A, $P<0.01$, Mann-Whitney U test).

Figure 1

Changes in equilibrium function: To estimate equilibrium function, the amount of time that rats remained on an accelerating rotating rod under a gradual increase in speed from 2 to 30 m/min for 5 minutes was measured. The average duration in 3 sessions was defined as rotarod performance. In MS rats, the relative values of rotarod performance compared to those at pre-surgery decreased to 0.6 ± 0.1 (mean ± S.D.) and 0.8 ± 0.2 on the 2^{nd} and 7^{th} days after surgery, respectively (Fig. 1B). Significant decreases were recognized on the 2^{nd} and 7^{th} days after surgery compared with pre-surgery ($p<0.01$, two-way repeated measures analysis of variance and Dunnett's test). In sham rats, however, no significant changes in rotarod performance were recognized after surgery. The relative values of rotarod performance for MS rats were significantly smaller than those for sham rats on the 2^{nd} and 7^{th} days after surgery (Fig. 1B, $p<0.05$, unpaired t-test).

Lactate threshold (LT): The LT was determined using software from individual plots of blood lactate concentration vs. treadmill speed. In an example recorded at pre-

surgery (Fig. 1C), the treadmill speed was increased by 5 m/min relative to that in the previous session, and no obvious increase in blood lactate concentration was recognized until 25 m/min. However, a rapid increase in the lactate concentration was observed at 30 and 35 m/min. In this case, LT was considered to be 25.4 m/min with the use of the software. Similarly, LTs on the 2nd and 7th days after surgery were determined to be 20.9 and 25.2 m/min, respectively (Fig. 1C, closed symbol).

The average values for the treadmill speed at LT in MS and sham rats are shown in Table 1. In MS rats, the average treadmill speed at LT significantly decreased on the 2nd day after surgery compared with pre-surgery (Table 1, $p<0.01$, two-way repeated measures analysis of variance and Dunnett's test). In sham rats, significant changes in treadmill speed at LT were not recognized after surgery. The average blood lactate concentrations at LT in MS and sham rats are shown in Table 1. There were no significant changes in blood lactate concentration at LT in MS or sham rats. Table 1

The relative changes in LT are shown in Fig. 1D. In MS rats, the relative value of LT on the 2nd day after surgery was significantly lower than that at pre-surgery (Fig. 1D, $p<0.05$, two-way repeated measures analysis of variance and Dunnett's test). On the 7th day after surgery, the relative value of LT recovered in comparison to that on the 2nd day, but tended to be lower than that at pre-surgery ($p<0.1$). In sham rats, no significant difference was recognized in the relative value of LT after surgery. The relative values of LT in MS rats on the 2nd and 7th days were significantly lower than those in sham rats (Fig. 1D, $p<0.05$, unpaired t test).

DISCUSSION

In the present study, apparent neurological deficits were observed in animals with microsphere (MS) induced-infarction, while most of the animals in the sham group did not exhibit any neurological deficits. A previous study similarly reported that microsphere-injected rats exhibited marked neurological deficits using the same criteria [13]. While these neurological deficits disappeared on the 7th day after surgery, rotarod performance of MS rats was significantly impaired after surgery in comparison

with sham rats. Rotarod performance is considered to reflect equilibrium function and the ability to perform coordinated movement [19]. Miyake et al. [14] reported that striatal blood flow on the microsphere-injected side was decreased on the 3rd day after surgery, and this was maintained up to the 14th day after surgery. Ischemia of the striatum elicits impairments in smooth motion and equilibrium function [20]. The energy expenditure during treadmill exercise in MS rats, compared with that in sham rats at the same speed, could be higher due to such impairments in smooth motion and equilibrium function. Most stroke patients show impaired equilibrium function and unstable gait patterns [11]. Energy expenditure in stroke patients was higher than that in healthy subjects during walking at a slow speed [12]. Thus, impairments in smooth motion and equilibrium function might cause a rapid increase in the blood lactate concentration during treadmill exercise even at low speed.

We have demonstrated that the LT in the acute period of cerebral infarction was significantly lower than that in sham-operated rats. In stroke patients, however, the stress of exercise is contraindicated in the acute period, and thus little is known about the changes in the LT. On the other hand, Ivey et al. [7] reported that the LT of stroke patients in the chronic period was low compared with to that of healthy subjects. It is known that the number of slow muscle fibers, which exhibit a highly oxidative metabolism, decreases because of disuse atrophy in the chronic period of stroke [21]. Lactate levels are known to be influenced by the muscle fiber composition [22]. Previous studies have reported that there is a strong relationship between the number of slow muscle fibers and the LT [1]. Muscle atrophy is usually not elicited within a few days after stroke, and thus the decrease in LT demonstrated in the present study should not be due to a decrease in the number of slow muscle fibers.

Choe et al. [23] reported that slow muscles exhibited significant atrophy compared to control rats on the 7th day after cerebral infarction induced by middle cerebral artery occlusion (MCAO). Ding et al. [24] reported that MCAO rats exhibited severe neurological deficits compared with sham-operated rats for up to 28 days after surgery. MCAO-induced cerebral infarction is considered to result in severe neurological deficits compared with cerebral infarction induced by MS injection. Thus,

rats that are used in the MCAO method might suffer slow muscle atrophy because of these severe neurological deficits.

It is known that the blood lactate concentration rapidly increases when an increase in exercise intensity leads to an oxygen deficit. In a previous study, maximal oxygen consumption in stroke patients during incremental exercise was lower than that in healthy subjects [7]. A decrease in respiratory function causes a rapid increase in the blood lactate concentration even at a low work rate; i.e., a decrease in LT [25]. Furthermore, a strong correlation has been observed between the plasma catecholamine and lactate levels during incremental exercise [26]. The stress produced in rats that are forced to run on a treadmill might produce a lot of catecholamine, and as a result the blood lactate concentration could increase even under relatively low-level exercise.

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Figure legends

Fig. 1 (A) Changes in neurological deficits caused by surgery. (B) Changes in rotarod performance caused by surgery. (C) Changes in the blood lactate concentration during forced running at pre-surgery, and on the 2^{nd} and 7^{th} days after surgery. Closed symbols indicate the LT determined by software. (D) Changes in the lactate threshold caused by surgery.

##p<0.01: There was significant difference on the 2^{nd} and 7^{th} days after surgery compared with the day after surgery in the same group (Friedman's test and Steel-Dwass test). ++p<0.01: there was a significant difference on the 2^{nd} and 7^{th} days after surgery compared with pre-surgery in the same group (two-way repeated measures analysis of variance and Dunnett's test). *p<0.05, **p<0.01: there was a significant difference between MS rats and sham rats (Fig. 1A; Mann-Whitney U test, Fig. 1B and D; unpaired t test).