A study of the relationships of changes in pain and gait after tourniquet-induced ischemia-reperfusion in rats

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Abstract. [Purpose] The purpose of this study was to determine the relationships of changes in pain and gait after ischemia reperfusion was induced by tourniquet in rats. [Subjects and Methods] The subjects were six ten-week-old male Wistar rats. Ischemia was induced in the left lower limbs of the experimental rats at a pressure of 300 mmHg for 90 minutes. Pain behavior evaluations were measured using the von Frey test in all the rats’ hind limbs. A consistently increasing plantar stimulus was applied until the rats exhibited an escape behavior. For the evaluation of gait, a two-dimensional motion analysis system was used to measure the distance from the calcaneus to the floor (DCF) and toe extension angle (TEA) during gait. The evaluations were performed in the normal state, 3 hours after ischemia-reperfusion, and daily until 7 days after ischemia-reperfusion. [Results] Compared with the normal state, the means of the pain threshold showed a significant decrease until 4 days after ischemia. In addition, both TEA and DCF continued to show a significant decrease at 7 days after ischemia as compared with the normal state. [Conclusion] This study revealed that hyperalgesia occurs after ischemia-reperfusion, and recovery of hyperalgesia occurred earlier than gait dysfunction recovery.

Key words: Tourniquet, Pain, Gait

INTRODUCTION

A tourniquet is a device consisting of a pressurizing device and rubber cuff to cut off the blood flow by air pressure1). A tourniquet is used to control bleeding during orthopedic surgery, such as total knee arthroplasty (TKA)1). The use of tourniquets on the limbs can produce ischemia-related changes in peripheral sites rather than in the cuff-attached site2, 3). In addition, an ischemia-reperfusion injury may also occur when the blood flow resumes4, 5). In ischemia-reperfusion injury, edema and inflammation may cause damage or degeneration of the tissue4, 6). In addition, the occurrence of pain and gait disturbance after ischemia-reperfusion has been confirmed in both laboratory animals and humans2, 11). In recent years, it has become customary to begin rehabilitation the early postoperative period aiming to achieve early discharge. For early discharge, the
recovery of gait is important. Postoperative early motor function is affected by pain, and pain often becomes an inhibiting factor in rehabilitation. Therefore, knowledge of the change in gait and pain after ischemia-reperfusion is clinically useful. Previously, many studies have investigated the effects ischemia-reperfusion has on the organism. However, the relationship of the changes in gait and pain after ischemia-reperfusion has not been clarified. It is important to know these relationships for prognosis prediction and effective treatment in postoperative patients. Accordingly, the purpose of this study was to determine the relationships of changes in pain and gait after ischemia-reperfusion was induced by tourniquet in rats.

SUBJECTS AND METHODS

Six 10-week-old male Wistar rats with a body weight of 327.0 ± 6.9 g (average ± SD) were used. The animals were housed in a temperature-controlled room at 23 °C on a 12-hour light-dark cycle, and were given free access to standard rat food and water. This study was performed according to the Prefectural University of Hiroshima’s Regulations on Animal Experiments and was approved by the Animal Experiments Committee (Approval Number: 13MA007).

Ischemia was induced with a DC1.6 tourniquet finger cuff, and a Rapid Cuff Inflator with an AG101 air source (D.E. Hokanson, USA) was used to apply pressure. The rats were anaesthetized with inhalation anesthesia and sodium pentobarbital (40 mg/kg b.w.t), and ischemia was induced in the left lower limb at a pressure of 300 mmHg for 90 minutes. After ischemia was completed, the tourniquet cuff was promptly removed, and the rats were returned to their cage. Pain behavior evaluation was measured using the von Frey test for the rats' hind limbs. The rats were initially moved to the stimulus cage, where they rested for 20 minutes. Then, a consistently increasing plantar stimulus was applied until escape behavior was exhibited. The range of the stimulation intensity was 0–50 g, and it was gradually increased by 2 g/sec. The stimulus intensity when the escape behavior appeared was recorded as the pain threshold. The pain threshold was measured 3 times, and the mean value was used in the analysis. The length of each stimulus was more than 5 seconds.

In the two-dimensional motion analysis of gait, an Exer-3/6 treadmill for rats and mice (Columbus, Inc., USA) was used. The treadmill was set at a speed of 10 m / min and an inclination of 0 °. Before the video recording, the calcaneus and the fourth metatarsal of the left hind limb were marked. A video recording was performed from the left side of each animal’s gait on the treadmill. A digital video camera (HDC-HS9-N, Panasonic®) was fixed in the sagittal plane 1 m away from the treadmill for the recording. The gait of each rat was recorded for 2 minutes. Toe extension angle (TEA) and the distance from the calcaneus to the floor (DCF) were measured on video images using computer software (Image J 1.44p, U.S. National Institutes of Health, Bethesda, MD, USA). TEA was defined as the angle formed between the line connecting the fourth metatarsal to the calcaneus, and the horizontal line just before the toe-off. DCF was defined as the distance between the calcaneus and the floor when the foot was flat. The data are expressed as the mean ± standard deviation analyzed using the Shapiro-Wilk test, and the normality of the pain threshold and the DCF data was confirmed. Repeated measures one-way analysis of variance was used for analysis of these measurements. The Dunnett test was used for the post-hoc comparison. The TEA data was not normally distributed, so the Friedman test was used for the analysis of TEA, and the Steel test was used for the post-hoc comparison. Statistical analyses were performed using Excel Statistics 2010 (Social Survey Research Information Co., Ltd., Tokyo, Japan). Significance was accepted for values of p<0.05.

RESULTS

The mean pain threshold values are shown in Table 1. A significant change in the pain threshold was confirmed at 3 hours after ischemia-reperfusion. Compared with the normal state, a significant reduction in the pain threshold was observed up to 4 days after ischemia-reperfusion (p<0.05). The means of TEA and DCF are shown in Table 2. Compared with the normal state, significant reductions in both TEA and DCF were confirmed from 3 hours after ischemia-reperfusion. Moreover, this significant reduction continued throughout the 7 days after ischemia-reperfusion (p<0.05). The tendency for recovery with the passage of time after ischemia-reperfusion was confirmed with each measurement.
DISCUSSION

This study aimed to determine the changes in gait and pain after ischemia-reperfusion was induced by tourniquet in rats. Ischemia elicits the production of inflammatory mediators, such as prostaglandins and inflammatory cytokines. Reperfusion also elicits the release of these chemical mediators into the blood and together, ischemia and reperfusion result in edema, inflammation, pain and hyperalgesia\(^5, 8, 9, 22\). Clinically, it has been reported that surgery without tourniquet results in less use of analgesics\(^23\). Generally, in animal studies, it is necessary to assess pain by evaluating pain-related behaviors\(^24\). In this study the von Frey method of stimulation with a 0.5 mm filament was used. The pain threshold was normal at 23.0 ± 4.1 g. It was reduced to about 56% of normal at 1 day after ischemia-reperfusion, and a significant reduction in the pain threshold was observed up to 4 days after ischemia-reperfusion (p<0.05). This result means that portions distal from the site of the tourniquet had become sensitive to stimulation after the ischemia-reperfusion. Pain is classified by the symptom, and it includes hyperalgesia and allodynia. In general, pain occurs when a stimulus is stronger than the pain threshold. In addition, pain is dependent on the stimulus intensity. Hyperalgesia is a stronger sensitivity to pain than the applied stimulus. On the other hand, allodynia is feeling pain with a weak stimulus that normally does not induce pain\(^24\). Hyperalgesia and allodynia often occur together. The von Frey method can be used to evaluate both hyperalgesia and allodynia. Changes in the pain threshold after ischemia-reperfusion may have been affected by the occurrence of both hyperalgesia and allodynia.

The results of the two-dimensional motion analysis show that there was a change in gait after ischemia-reperfusion. The normal state results of this study were TEA of 88.0 ± 2.9 ° and DCF of 15.6 ± 0.6 mm. The DCF results show that the calcaneus did not ground on the floor in normal rat gait. Also, in normal human gait, the vertical direction of the ground reaction force is maximum just before toe-off\(^25\). At this time, the toes are in hyperextension, and the load on the toes increases. The values of the TEA showed that the load increased on the toes in the normal rat gait.

The TEA and DCF values showed significant reduction after ischemia-reperfusion. From the results of the pain behavior evaluation, the occurrence of hyperalgesia and allodynia lasted up to 4 days after the ischemia-reperfusion. This is the state in which pain occurs with a weaker stimulus than usual. In addition, reduction of the TEA after ischemia-reperfusion could be regarded as a reduction of the load on the toes during toe-off. In other words, this result can be interpreted as reflecting pain-avoidance behavior. Therefore, the gait changes after ischemia-reperfusion were considered to reflect the change in the pain threshold. However, after 5 days after ischemia-reperfusion, both the TEA and DCF values were still significantly decreased compared with the normal state (p<0.05).

Iwata\(^21\) performed rat gait analysis and reported that muscle weakness is related to a decrease in DCF after muscle injury. Umei et al.\(^10, 14\) reported that gait disturbance and skeletal muscle damage occur after an ischemia-reperfusion pressure of 300 mmHg for 90 minutes. The present study also used the same tourniquet conditions; therefore, skeletal muscle damage might have occurred. Accordingly, it is possible that the gait changes after ischemia-reperfusion were related to a decrease in muscle contraction. However, in this study, the skeletal muscles, were not evaluated, so it will be necessary to evaluate skeletal muscle function in a future study.

This study investigated the relationship between gait and pain after ischemia-reperfusion. There is a possibility that allodynia and hyperalgesia were both involved in the gait changes seen up to 4 days after ischemia-reperfusion, and recovery of the pain threshold was faster than that of gait. Further studies should be carried out to clarify the other factors involved in gait changes after ischemia-reperfusion.

### Table 1. Pain threshold: stimulation intensity of the escape reaction in the von Frey method (mean ± SD)

| Evaluation time | Pain threshold (g) |
|-----------------|--------------------|
| Normal state    | 23.0 ± 4.1         |
| 3 hours         | 16.6 ± 4.5 *       |
| 1 day           | 12.8 ± 3.3 *       |
| 2 days          | 14.6 ± 2.2 *       |
| 3 days          | 15.5 ± 3.0 *       |
| 4 days          | 17.1 ± 2.9 *       |
| 5 days          | 19.5 ± 3.0         |
| 6 days          | 18.5 ± 2.2         |
| 7 days          | 21.5 ± 2.1         |

Evaluation time is the time elapsed after ischemia-reperfusion.
*p<0.05, Significant differences from the normal state

### Table 2. Results of the two-dimensional motion analysis (mean ± SD)

| Evaluation time | TEA (°) | DCF (mm) |
|-----------------|---------|----------|
| Normal state    | 88.0 ± 2.9 | 15.6 ± 0.6 |
| 3 hours         | 37.5 ± 7.4 * | 5.2 ± 1.1 * |
| 1 day           | 43.4 ± 8.5 * | 4.7 ± 1.0 * |
| 2 days          | 43.0 ± 9.3 * | 4.5 ± 1.6 * |
| 3 days          | 46.2 ± 9.5 * | 6.2 ± 1.0 * |
| 4 days          | 51.4 ± 5.4 * | 8.0 ± 1.3 * |
| 5 days          | 60.9 ± 7.2 * | 9.0 ± 0.8 * |
| 6 days          | 73.2 ± 9.5 * | 9.1 ± 1.3 * |
| 7 days          | 73.1 ± 4.7 * | 10.5 ± 0.5 * |

TEA: Toe extension angle just before toe-off; DCF: distance between the calcaneus and the floor when the foot is flat
*p<0.05, Significant difference from the normal state
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