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Original Article

Effects of physical exercise on the functional recovery of rat hindlimbs with impairments of the sciatic nerve as assessed by 2D video analysis

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Abstract. [Purpose] The purpose of this study was to investigate the effects of treadmill training on functional recovery by analyzing the ankle joint as well as the knee and hip joints with 2D video analysis during gait by rats with sciatic nerve injury. [Subjects and Methods] Twenty-four male Sprague-Dawley rats were used in this study. The sham group (SG) received only a sham operation without any sciatic injury; the training group (TG) performed treadmill training for 4 weeks after sciatic injury; and the control group (CG) wasn't provided with any therapeutic intervention after sciatic injury. [Results] The ankle, knee, and hip ROM of TG and CG during the initial, mid stance, and toe-off phases of gait at post-test were significantly different from SG. [Conclusion] Physical exercise, like treadmill training, is beneficial for the improvement of the ankle, knee and hip joints of rats with crushed sciatic nerve injury.
Key words: Sciatic nerve injury, Physical exercise, 2D video analysis

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INTRODUCTION

Peripheral nerve injury causes defects in the motor and sensory functions of the innervated region[1)]. The impaired axons of peripheral nerves have higher regenerative ability than those of the central nervous system. However, functional recovery is often incomplete, resulting in long-term dysfunction[2)]. Physical exercise is very important as a therapeutic tool for rehabilitation after nerve injury. Treadmill training performed after peripheral nerve injury is known to be effective for nerve regeneration and functional recovery[3)].

In studies of the peripheral nervous system, axon injury models of the sciatic nerve of rats have been widely used to investigate the effectiveness of diverse therapeutic strategies, such as medicine and training[4, 5)]. Although motor and sensory tests have been used to quantify the functional recovery of axon injury, there are still no measurement tools for functional recovery[6)]. Thus, valid and reliable functional tests are needed for the evaluation of the effectiveness of diverse treatment strategies. Recently, kinematic parameters have been used as an investigation technique to evaluate functional nerve recovery after sciatic nerve[7)].

A crushed sciatic nerve might influence kinematic changes not only at the ankle joint but also in the whole hindlimb during gait[8)]. However, previous studies have focused on changes at the ankle joint after sciatic nerve injury and no study has yet investigated the whole hindlimb function together with the knee joint and hip joints after physical exercises using 2D video analysis. Thus, the purpose of this study was to investigate the effects of treadmill training on functional recovery by analyzing the ankle joint as well as the knee and hip joints with 2D video analysis during gait by rats with sciatic nerve injury.

SUBJECTS AND METHODS

Subjects

Twenty-four male Sprague-Dawley rats weighing 250–300 g and aged 8 weeks old were used in this study. All animals were housed under conditions (4 rats per cage) of 23±2 °C and 50±5% humidity with a 12 hour light/dark cycle. The rats were given a 5-day period for adaptation to the environment before the experiment. Pellet feed and water were freely provided to the rats during experimental period. The experimental procedure was in complied with the management guideline for experimental animals and this study was approved by Animal Care and Use Committees of Daegu University. The rats were divided into the following three groups: the sham group (SG) which received only a sham operation without any sciatic injury; the training group (TG) which performed treadmill training for 4 weeks after sciatic injury; and the control group (CG) which was provided with no therapeutic intervention after sciatic injury. All surgical procedures and experimental protocols followed

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Daegu University's guidelines and were approved by the Institution of Animal Care and Use Committee (IACUC).

Methods

Zoletil (1 ml/kg) and Rompun (1 ml/kg) were injected into the abdominal cavity in order to anesthetize the rats. After checking for anesthesia using the avoidance reaction from pain, the rats were fixed on the operating table. To create the sciatic nerve injury, the skin between the right thigh and knee joint was shaved, a skin incision of 2 cm was made at the shaved area, and the sciatic nerve was isolated from the surrounding muscles. The proximal area 7 cm from the ankle joint and just before the bifurcation point of the tibial and peroneal nerves was compressed for 30 seconds using haeomostatic forceps. The crushing was conducted after sterilization with 70% alcohol of the forceps which were covered with soft plastic material in order to prevent damage by the forceps. The forceps used in this study were designed to provide 3 classes of force, and the end of forceps was marked in order to provide regular pressure. After compression, the skin was closed by suture using suture thread for animals, and sterilization was then performed again to prevent infection[9].

The rats in TG had a 1-day break after sciatic nerve injury, then they performed treadmill training 5 times a week for 4 weeks. A treadmill for small animals (JD-A-09 type, JEUNGDO Bio & Plant Co., Ltd., Korea) was used in this study following a modification of Chuna's method[10]. The rats trained for 20 minutes at 5 m/min during the first week, and then for 1 hour at 15 m/min for the following 3 weeks. The rats were induced to continually run on the treadmill by low intensity (<2.0 mA) electric shocks delivered at the end of the treadmill.

Motion analysis using the Dartfish program was conducted on the first and 28th days after the sciatic injury in order to analyze the kinematic changes occurring during recovery from sciatic nerve injury. Before video recording, 70% of the hindlimb was shaved after alcohol sterilization. The hip joint was defined by the beaky angle between the pelvis and femur. The pelvis angle was defined by the line connecting the markers at the anterior superior iliac spine and ischial tuberosity, and femur orientation was provided by the segment defined by markers at the greater trochanter and the lateral aspect of the knee. The knee angle was defined by caudal the angle between the femur and the leg, denoted by markers at the knee and lateral malleolus. The ankle joint was defined as the angle formed between the leg and hindfoot, defined by markers at the lateral malleolus and head of the fifth metatarsal. The ankle's zero degrees position accorded with the angle between the leg and hindfoot. Ankle dorsiflexion and plantarflexion angles were then positively and negatively measured[11]. Joint angle was measured at initial contact, mid stance and toe-off. Video recording was performed during gait in a transparent passage 100 cm in length, 8 cm in width and 10 cm in height. The rats were encouraged to move forward by setting up a dark area at the end of the passage. Before recording, each rat was allowed to walk the passage 3 times to familiarize them with the direction of passage; then, the gait of each rat was recorded repeatedly three times. The 60 Hz digital video camera was fixed in the sagittal plane 1 m away from the passage for the recording.

One-way ANOVA was performed using PASW 18.0 for Windows in order to investigate the joint angle changes of the ankle, knee and hip joints between the first day and 4 weeks after the sciatic injury. LSD was used for post hoc comparisons. The significance level was chosen as 0.05.

RESULTS

ROM of the ankle joint is shown in Table 1. The ankle ROM of TG and CG during initial contact at pre-test were significantly different from that of SG. The ankle ROM of TG and CG during mid stance at pre-test were significantly different from that of SG. The difference between post and pre-test in TG was significantly different from CG and SG during toe off.

ROM of the knee joint is shown in Table 2. The knee ROM of TG and CG during initial contact at pre test were significantly different from that of SG, but at post-test, knee ROM of SG and TG were significantly different from that of CG. The knee ROM of TG and CG during mid stance at pre-test were significantly different from that of SG. In addition, the knee ROM of TG and SG during toe off at post-test were significantly different from that of CG.

ROM of the hip joint is shown in Table 3. The hip ROM of TG and CG during initial contact at pre-test were significantly different from that of SG. The hip ROM of TG and CG during mid stance at pre-test were significantly different from that of SG. In addition, the hip joint ROM of TG and CG during toe off post-test were significantly different from that of SG.

DISCUSSION

In this study, the degree of recovery of the hip joint, knee joint and ankle joint during gait was evaluated using 2D video analysis after rats with sciatic nerve injury had performed the treadmill training in order to investigate the effects of physical exercise on the functional recovery of rats with impairment of a peripheral nerve.

Although there are some methods of analyzing the joint kinematics during the gait of rats, 2D video recording is a method which demonstrates good day to day and inter-observer reliabilities[12]. The Dartfish program for 2D video analysis is widely used to analyze not only the gait of rats, but also the movements of athletes, and it is well known as a highly reliable and valid method. Therefore, we used the Dartfish program to measure the hip, knee and ankle kinematics during the gait of rats after experimental sciatic nerve crush injury.

The results of this study show that the angle of dorsiflexion in CG and TG tended to significantly increase, compared with SG at pre-test. The sciatic nerve provides the motor and sensory innervations of the hindleg and foot, and impairment of this nerve is known to cause the denervation of muscles related to the ankle joint[13]. It is possible that this denervation increased the angle of dorsiflexion in CG and TG. This result is in agreement with previous studies of experimental sciatic nerve crush injury[11]. At post-test, there was a significant difference in ankle dorsiflexion between CG and SG, but not

Table 1. Comparison of ankle joint angles among the groups (unit: °)

	Initial contact			Mid stance			Toe off		
	Pre	Post	Post-Pre	Pre	Post	Post-Pre	Pre	Post	Post-Pre
SG	−12.95±5.28[a]	−15.70±5.45	−2.75±8.16	0.30±5.77[a]	−1.70±5.68	−4.38±5.64	−14.59±6.49[a]	−15.43±6.03	0.41±9.50
CG	6.03±6.32	−8.78±9.31	−14.80±10.40	32.41±6.39	18.70±8.04[b]	−13.71±10.59	24.96±6.33	19.05±3.73[b]	−5.91±6.97
TG	6.67±7.57	−20.30±12.18	−34.98±8.31[c]	37.14±6.29	−0.33±2.66	−37.46±6.64[c]	23.03±8.69	−11.55±11.46	−42.78±7.76[c]

[a] significantly different from CG and TG
[b] significantly different from SG and TG
[c] significantly different from SG and CG

Table 2. Comparison of knee joint angles among the groups (unit: °)

	Initial contact			Mid stance			Toe off		
	Pre	Post	Post-Pre	Pre	Post	Post-Pre	Pre	Post	Post-Pre
SG	109.71±10.61[a]	107.41±10.80	−6.05±5.84	87.73±11.69 [a]	83.10±11.78	−12.13±11.48	86.55±12.87	86.81±12.95	−2.73±12.87
CG	133.75±13.35	128.13±16.06 [b]	−5.63±16.73	104.48±12.95	92.54±8.07 [b]	−11.94±12.60	96.14±16.25	92.15±13.86[b]	−3.99±12.25
TG	126.33±12.56	104.65±11.91	−29.18±17.20[c]	102.73±7.15	73.20±5.92	−29.53±6.11[c]	99.05±5.60	73.93±5.90	−25.13±9.56 [c]

[a] significantly different from CG and TG
[b] significantly different from SG and TG
[c] significantly different from SG and CG

Table 3. Comparison of hip joint angles among the groups (unit: °)

	Initial contact			Mid stance			Toe off		
	Pre	Post	Post-Pre	Pre	Post	Post-Pre	Pre	Post	Post-Pre
SG	66.30±5.21[a]	63.48±6.88	−6.58±5.75	64.40±11.46 [a]	68.20±5.67	3.80±13.65	71.45±12.69 [a]	70.74±8.27	−0.71±8.80
CG	79.18±9.86	82.18±4.99[b]	3.00±13.21	90.83±8.59	81.65±7.27 [b]	−9.17±10.90	106.00±9.29	89.45±5.22 [b]	−16.55±5.73
TG	76.30±16.73	70.45±8.57	−4.60±15.29	100.55±7.17	73.13±10.59	−32.42±4.50 [c]	102.48±10.38	72.81±11.80	−29.66±14.66[c]

[a] significantly different from CG and TG
[b] significantly different from SG and TG
[c] significantly different from SG and CG

between TG and SG. Furthermore, the difference between post and pre-test in TG showed a statistically significant increase in plantar flexion, compared to the other groups. Ankle joint motion is a valid and reliable method of evaluating not only sciatic nerve injury, but also reinnervation and functional recovery[14, 15]. Based on this method, the post-test and post-pre difference values might indicate that treadmill training decreases dorsiflexion.

There were also statistically significant differences in hip and knee joint angles. Previous studies of experimental sciatic nerve crush have focused on the evaluation of the ankle joint. However, sciatic nerve injury can affect the kinematic changes of the whole hindlimb including the knee and hip[8]. The change of normal patterns in the knee and hip joints during gait is the result of two mechanical changes. The first is the change in central control caused by motor and proprioceptive deficits in the muscles, and the second is the loss of motion at the ankle and foot[16]. Generally, the ankle joint provides load bearing control and joint stability during the stance phase. However, after sciatic nerve injury, the ankle joint cannot contribute to this control anymore, and the other hindlimb joints such as the hip and knee joints compensate for this function. The increases of extension in the hip and knee joints during the stance phase help to place the soles fully in contact with the floor by maintaining the length of the lower limbs[11]. We think that this compensation is the reason why the extension values at pre-test in CG and TG were significantly higher than in SG. The value of extension more significantly deceased between post- and pre-test in TG than in CG. It seems that the compensation mechanism was reduced by increase of plantar flexion at the ankle joint. Thus, we consider treadmill training is beneficial for the improvement of normal functional recovery of the hip and knee joints.

Treadmill training can enhance myelinated fiber maturation and increase the area of myelinated fibers in rats with sciatic nerve injury. In addition, the level of muscle reinnervation and the number of regenerated myelinated axons are increased by treadmill training[17, 18], leading to the functional recovery of the hindlimb and nerve regeneration[3]. We think this is the reason for the improvements observed in the ankle, knee and hip joints in TG during gait.

Based on the results of this study, we consider kinematic analysis using 2D video recordings is a useful method for analyzing sciatic nerve injury. In addition, physical exercise like treadmill training is beneficial for the improvement of functional recovery not only for the ankle joint, which has been the focus of previous studies, but also of the knee and hip joints of rats with sciatic nerve injury.

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