Treadmill Exercise Facilitates Functional Recovery and Neuroprotection in Subacute Stroke Rat

J. Sun, K. Y. Tong, Z. Ke, S. J. Zhang, X. L. Hu, and X. X. Zheng

***Abstract*—Treadmill exercise has been a controversial method to help stroke rat with functional recovery and neuroprotection. In this study, we investigated the effect of treadmill exercise on focal brain ischemia stroke rat in subacute phase. Twelve rats were induced focal ischemic stroke by a 60 min middle cerebral artery occlusion/reperfusion surgery and then randomly divided into two group, control group (CG, n=6) and treadmill group (TG, n=6). TC rats received daily treadmill exercise training during the seven-day experimental period while CG rats did not receive any intervention. Daily functional test results were recorded and all rats were sacrificed for TTC staining on the 7^{th} day. The increase rate of behaviour score in TG is significantly higher than that in CG (*P*<0.05) and the infarct volume in TG is significantly smaller than that in CG (*P*<0.05). Results illustrated that post-stroke treadmill exercise can facilitate the functional recovery and provide neuroprotection in focal ischemic stroke rat in the subacute phase.**

***Index Terms*—Focal ischemic, MCAo, stroke rat, treadmill exercise.**

I. Introduction

Stroke has been reported to be the third life killer in the world beyond cancer and heart disease [1] and the leading cause to make the suffered disabled [2]. Investigation has been made for many years to find out the mechanism of cell death when stroke happens [3]-[5] methods for treatment and ways to facilitate motor function rehabilitation [2], [6]. Due to the increasing number of people in the world, the high possibility exists that more people may suffer from post-stroke disability. According to the Hong Kong Authority Statistical Report 2009-2010, the number of annual stroke admissions to public hospital has been increasing from 24,742 cases in 2005 to 25,614 in 2009 [7]. It's a huge burden not only to the whole society but to the families with stroke patients. Thus, it is in great demand to find out a better way to help the disabled recover their motor function for regaining self-living skills to relieve the burden of the society and their families. Treadmill exercise, a kind of conventional and easy-to-think-of means, has been used and studied [8], [9] for many years. It is universally used in the subacute stroke period because this phase is the golden period for treatment and recovery [10]. However, in recent years, post-stroke treadmill in this subacute phase was suggested to bring about more brain damage. Treadmill exercise is a forced exercise in animal studies which may induce stress, and stress may have negative effect on rats' recovery, since stress has been found to affect structural plasticity of the hippocampus in two forms [11]: repeated stress causes atrophy of dendrites in the CA3 region, and both acute and chronic stress suppresses neurogenesis of dentate gyrus granule neurons. On the contrary, other researchers believed treadmill exercise training can protect neurons [12], [13]. For example, Hayes' study showed that treadmill exercise assisted neuroprotection and it was proposed that there is something else brought by treadmill training but not the stress inducing such neuroprotection result [14]. Such controversial outcomes brought by treadmill may be caused by species differences of the rats, the experimental operation. Because of the application value, whether post-stroke treadmill exercise training in subacute phase can facilitate motor functional recovery and protect neurons needs to be further investigated with more studies. In this study, we investigated the effect of treadmill training on motor function recovery and neuroprotection in subacute phase using rat model.

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J. Sun, K. Y. Tong, Z. Ke, S. J. Zhang, and X. L. Hu are with the Interdisciplinary Division of Biomedical Engineering, The Hong Kong Polytechnic University, Hong Kong, China, SAR (e-mail: jing.cc.sun@connect.polyu.hk, k.y.tong@polyu.edu.hk, ashtar_22@hotmail.com, zjusolo1985@gmail.com, xiaoling.hu@polyu.edu.hk).

X. X. Zheng is with the Department of Biomedical Engineering and Instrument, Zhejiang University, Hangzhou, Zhengjiang, China (e-mail: zxx@mail.bme.zju.edu.cn).

II. Methodology

A. Intraluminal Suture Middle Cerebral Occlusion

Twelve male Sprague-Dawley (SD) rats with body weight of 280-320g provided by the Central Animal Facility of the University were used in this study. All rats were allowed free access to food and drinks throughout the whole period.

Before being induced intraluminal suture middle cerebral artery occlusion and reperfusion (MCAo/r) surgery, all rats would go through a 3-day accommodation, i.e., running on the treadmill (KN-73, Natsume Ltd., Japan) for 10, 20, and 30mins at a velocity of 25m/min and with an inclination of 0 degree daily. This was aimed to minimize the stress rats would meet when placed in a strange situation. After accommodation, all rats were given MCAo/r surgery to induce stroke. This focal ischemic stroke rat model was first introduced by Longa *et al* in 1989 and has been improved and widely used over the decades [15]. In this study, the surgery process was the same as that by Z. Ke *et al.* [16]. Briefly, rats were anesthetized by 10% chloral hydrate (0.4mg/kg for induction and 0.02mg subsequently). Afterwards, incisions were made at the midline of the neck to expose the common

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carotid artery (CCA), and then the external carotid artery was found and ligated by a cotton tread. Subsequently, a commercial filament with the tip diameter of 0.38±0.02mm (Beijing Sunbio Biotech, China) was inserted into the CCA and advanced along the internal carotid artery until the tip of the filament reached the middle cerebral artery. The occlusion lasted for 60 min and the filament was then withdrawn to allow reperfusion. After the surgery, all rats were induced stroke and were randomly divided into two groups, control group (CG, n=6) and treadmill group (TG, n=6).

All procedures performed were approved by the Animal Ethics Review Committee of the University and conformed to international guideline on the ethical use of animals.

B. Treadmill Protocol

After the surgery, all rats in CG and TG would go through a seven-day experimental period with or without treadmill exercise respectively. Specifically, rats in CG were housed in standard cage, while rats in TG were forced to run 30min on the treadmill at the same velocity. During the 30 minutes' exercise, rats were allowed 10 minutes' break after each 10 minutes' running. Fig. 1 shows the experimental setup for the rat running on the treadmill.

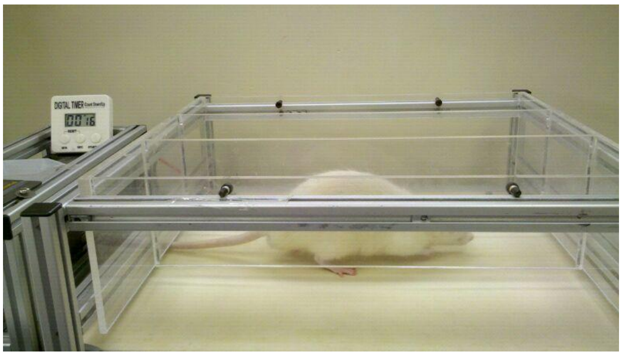

Fig. 1. An experimental rat was running on the treadmill.

C. Behaviour Tests

Since rats will get sensorimotor deficits after surgery, neurological evaluation was performed according to the protocol of De Ryck. Specifically, through the seven days, the behaviour score of the rats were tested daily to evaluate the motor function recovery. According to De Ryck's behavioural test, 6 tasks including 8 subtasks were required and up to 16 points were given [17]. The score range for each subtask is 0 to 2, and the higher score indicates the better motor function.

D. 2, 3, 5-Triphenyltetrazolium Chloride (TTC) Staining

The brain infarct volumes which indicate the brain damage level were obtained by 2, 3, 5-triphenyltetrazolium chloride (TTC) staining method. TTC staining validity depends on mitochondrial enzyme damage. In normal tissue, TTC reduces by mitochondrial enzyme and these brain regions appear brick red; while in infarct area, no reaction happens and it results in white after staining. In this study, after 7 days' intervention, rats were sacrificed and their brains were obtained and sliced in to 2mm by a brain matrix and then immersed in 2% TTC solution for 30 minutes. When staining was completed, a digital camera was used to record the staining results. Finally, infarct volume was calculated by using ImageJ [18].

E. Statistical Analysis

The results were expressed as means ± SD. MATLAB (version 7.11.0.584) was used for data analysis in this study. T-test was used to analyse whether there was significant difference between the two groups.

III. Results

The body weight gradually increased over the experimental period in both the two groups, as shown in Fig. 2. The mean body weight in CG was higher but not significant than that in TG. This may be due to that treadmill is an energy-consuming exercise.

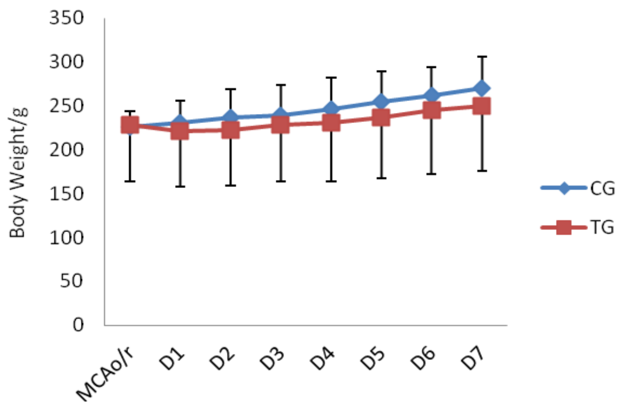

Fig. 2. Body weight changing trend in the two groups. Both of CG and TG went through an gradually increacing trend, while the CG was higher than the TG.

A. Motor Function Recovery

Rats had different initial stroke levels, in this study, thus the relative recovery rates were calculated and compared between the two groups. Fig. 3 showed the relative values of behaviour scores by comparing daily behaviour score with that in the first day. Results showed that, all rats will spontaneously and gradually recover their motor function in the subacute period. However, according to the increasing rate of behaviour score, rats with daily treadmill exercise recovered significantly faster than those without any intervention (P<0.05) over the seven subacute days.

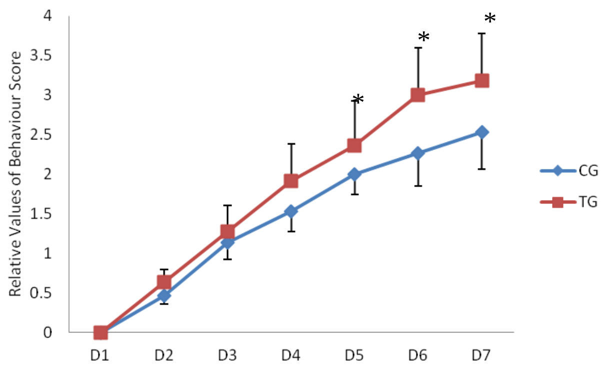

Fig. 3. The trend of relative values of behaviour scores in CG and TG over 7 days (* p<0.05).

B. Neuroprotection

Fig. 4 is an example of TTC staining results in CG (Left) and TG (Right). The white region means the neurons in this area died, i.e., the infarct area, while the brick red region indicates normal brain tissue. All rats' infarct volumes were calculated. The mean infarct volumes in CG and TG were 282.306 ± 21.961 mm^3 and 202.08 ± 39.854 mm^3 respectively, shown in Fig. 5. Results showed that the infarct volume in TG was much smaller than that in CG (P<0.05), which demonstrated that post-stroke treadmill exercise in the subacute phase may contribute to the neural protection.

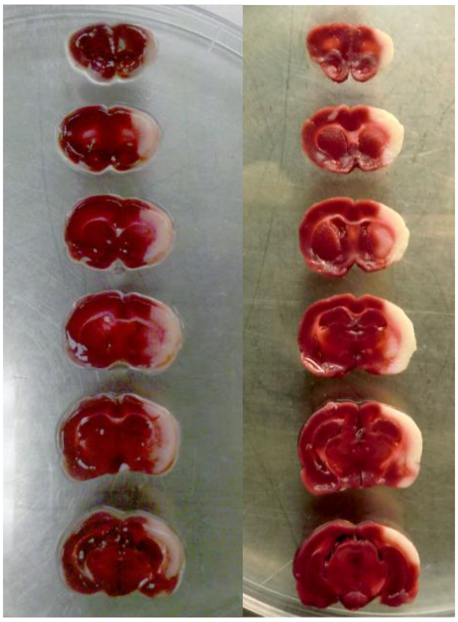

Fig. 4. An example of TTC staining results in CG (Left) and TG (right) respectively. The white region represents the infarct area where neuron died, while the brick red means normal brain tissue. Part of the striatum and ipsilateral motor sensory cortex were the targeted brain area.

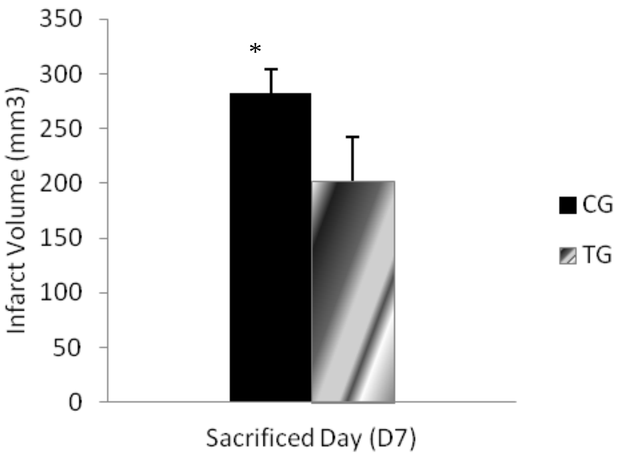

Fig. 5. Infarct volume of the two groups. The mean infarct volume in CG and TG were 282.306 ± 21.961 mm3 and 202.08 ± 39.854 mm3 respectively. T-test results showed that there was a significant difference between them (P<0.05), which may reveal the neuroprotection effect of post-stroke treadmill exercise in the subacute phase.

IV. Conclusion

Twelve adult SD rats were used to investigate the effect of post-stroke treadmill exercise on motor function recovery and neuroprotection. Since the initial stroke level is different, it may be convenient to consider the recovery rate by comparing the daily behaviour score with that in the first day. From the increasing speed of the behaviour score, it showed that stroke rats can obtain a relatively faster motor function recovery rate if they were forced to run on the treadmill for 7 consecutive days through a subacute stroke period. The infarct volume on the 7^{th} day was calculated through TTC staining. The mean infarct volume was smaller in TG than that in CG (P<0.05), which may indicate that post-stroke treadmill exercise in subacute phase can protect neurons from dying to some extent or it may facilitate neural regeneration.

V. Discussion

Treadmill exercise training as a means of motor function recovery has been applied widely and clinically and its effect has been studied for many years [8], [9]. However, its effect on motor function recovery and neuroprotection was controversial in recent years. Possible reasons are the different protocols, intervention starting point and sustained period, even the different operation, species and the considering angle. Due to the high application value, it is needed to clarify whether treadmill exercise training has positive effect in different stroke phases.

In this study, we considered male Sprague-Dawley rats weighing between 280g and 320g in the subacute stroke phase and results showed infarct volume was reduced compared to control group. Similar results have been found by Yang et al [10] when they investigated the effect of early and late treadmill training after focal brain ischemia in male Sprague-Dawley (SD) rats with an age between 2 and 3 months. They found that early treadmill training (started 24h post MCAo/r surgery, 30min per day, 5 days a week with a speed of 20m/min and 0° slope, and rats experienced one-week training and another one-week resting, and then were sacrificed) had significant effects in reducing brain infarct volume compared with spontaneous recovery. In Matsuda's study [13], researchers found that treadmill exercise starting from 24h after MCAo/r surgery for 28 consecutive days reduced neurological deficits and infarct volume in Wister rats weighing 220-260g. Even though the protocols (one-week training plus one-week resting, 4-week training, and this one-week consecutive training), the species (SD rats or Wister rats) or weights are different between theirs and ours, all results supported that treadmill training can facilitate post-stroke motor function recovery and provide neuroprotection in rats. This study also took motor function recovery rate as a new parameter to investigate the difference between TG and CG. In the future, training intensity and stress brought by treadmill training will be further studied to know more about the mechanism of treadmill training facilitating the recovery of stroke rats. This study lays more information for our further investigation.

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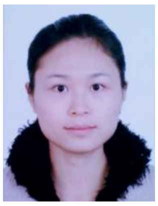
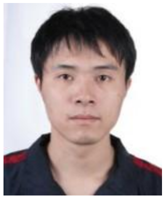


Jing Sun received the B.Sc. degree from the Department of Biomedical Engineering, Zhejiang University, in 2010. Now she is an M.Phil student in the Interdisciplinary Division of Biomedical Engineering, the Hong Kong Polytechnic University, with research interests in biomedical signal processing and stroke rehabilitation.

Kai-Yu Tong received his PhD in Bioengineering from the University of Strathclyde, Glasgow, UK in 1998. He joined the Hong Kong Polytechnic University in 1999, and now he is a professor in the Interdisciplinary Division of Biomedical Engineering in 2008. He is currently the program leader of the Biomedical Engineering undergraduate program. His research interests include rehabilitation robot, functional electrical stimulation, stroke rat model and BCI training rehabilitation on persons after stroke.

Zheng Ke is a research associate at the Hong Kong Polytechnic University. She received her PhD in Biomedical Engineering from Hong Kong Polytechnic University in 2012. Her research interests include stroke rehabilitation using animal models.



Shaojie Zhang received his M.Phil degree in Biomedical Engineering from The Hong Kong Polytechnic University, Hong Kong in 2012. He is currently a research assistant in the Interdisciplinary Division of Biomedical Engineering, the Hong Kong Polytechnic University, Hong Kong. His research interests include stroke rat models and biomedical signal processing.

Xiaoling Hu received the B.S. degree from the Department of Biomedical Engineering, Zhejiang University, in 1998. She obtained the MPhil and PhD degrees from the Department of Electronic Engineering, the Chinese University of Hong Kong, in 1999 and 2002 respectively. She is currently an instructor in the Interdisciplinary Division of Biomedical Engineering, the Hong Kong Polytechnic University, with research interests in neural engineering and intelligent devices for stroke rehabilitation.

Xiaoxiang Zheng received her B.E. in Radio Technology from Zhejiang University, Hangzhou, China, in 1968. She obtained her M.S. and M.D. in Basic Medical Science from Tsukuba University, Japan, in 1984 and 1993 respectively. Currently, she is the Full Professor of Biomedical Engineering and the Executive Director of Qiushi Academy for Advanced Studies of Zhejiang University. Her primary research interests include microcirculation, physiology of cell, drug screening and neural engineering.