Bull Yamaguchi Med Sch 64(3-4):21-26, 2017

An invited review following *the Soujinkai Fujiu Memorial Award*: Development of a Neurorehabilitation System for Effective Functional Recovery after Spinal Cord Injury: Approach to Clinical Application from Basic Research

Tsukasa Kanchiku, Hidenori Suzuki, Yasuaki Imajo, Atsushi Moriya, Yutaka Suetomi, Norihiro Nishida, Masahiro Funaba, Youhei Takahashi and Toshihiko Taguchi

Department of Orthopaedic Surgery, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-kogushi, Ube, Yamaguchi 755-8505, Japan
(Received October 5, 2017)
Correspondence to Tsukasa Kanchiku, M.D., Ph.D. E-mail: tkanchik@yamaguchi-u.ac.jp

Abstract Combination therapy is essential for functional repairs of the spinal cord. Rehabilitation facilitates the reorganization of residual/regenerated neural pathways and improves motor function after spinal cord injury (SCI). Functional electrical stimulation has previously been used as neuroprosthesis for quadriplegics and can be used in providing rehabilitative therapy to aid in the reorganization of central nervous system after spinal cord regeneration therapy. We have developed a rodent model of a less invasive neuromuscular electrical stimulation (NMES) capable of working in combination with other therapies. A series of studies using this model have indicated that NMES therapy is effective in recovering motor function after SCI. We have now developed a gait analysis system in collaboration with the rehabilitation division in our hospital. In future studies, we will test the efficacy of various methods of walk training in conjunction with this system to develop an effective rehabilitation support system that will maximize functional recovery for patients with lower limb paralysis.

Key words: spinal cord injury, neurorehabilitation, 3D kinematic analysis, functional electrical stimulation, body wight supported treadmill training

Introduction

Neuroprotection, axonal growth stimulation, lost tissue replacement, and axonal transmission enhancement are current areas of research; however, because each treatment has limitations when used alone, combination therapy is necessary.[1] We have previously conducted several spinal cord regeneration studies,[2-6] which showed that when used independently, these were ineffective in functional recovery after spinal cord injury (SCI).

The stimulation of the intrinsic plasticity of the nervous system via rehabilitation maximizes functional recovery after incomplete SCI (iSCI), and it is hoped that rehabilitation stimulates the reorganization of neural circuits after regenerative therapies.[1] In patients with SCIs, repeated rhythmical leg exercise, along with robot-assisted treadmill (TM) walking and functional electric stimulation therapy, is believed to be clinically effective.[7-9] Despite this, the mechanism underlying improvement in motor function remains unclear.[10] Basic research that includes animal experimentation is needed to completely understand the mechanism of functional recovery and to examine its effectiveness when combined with spinal cord regeneration therapies.

Jung et al. have previously created electric stimulation models using rats as a model of functional neuromuscular electrical stimulation (NMES) in which motor points of agonist muscles of limbs were stimulated using embedded electrodes. They have also performed a basic experiment to evaluate stimulation conditions required to initiate synkinesia in each joint during gait.[11-13] Significant short-term improvements in the hindlimb synkinesia of rats with iSCIs after NMES therapy were reported.[13] NMES therapy can be used in the acute stage post injury; however, Jung's model used embedded electrodes, making it a highly invasive procedure. We created a less invasive NMES therapy model using percutaneous needle electrodes and reported its effectiveness in functional motor recovery after SCI.[14] Here, we report a basic research series to establish an effective neurorehabilitation system after SCI and described the future prospects of our research.

Establishment of a Less Invasive NMES Therapy Model and a Series of Basic Studies

A series of studies were approved by the Committee for the Care and Use of Animals at the Yamaguchi University. The study was conducted in accordance with Yamaguchi University Animal Regulations; laws on the care and maintenance of laboratory animals (Act No. 105); standards for the care and maintenance of experimental animals for the reduction of their suffering (Ministry of the Environment Public Notice No. 88); and basic policies on the use of animal experimentation at research institutions (Ministry of Education, Culture, Science, Sports, and Technology Public Notice No. 71).

Less invasive NMES therapy model using needle electrodes[14]

Ten mature female Fischer rats (160-175 g) were used. Needle electrodes were inserted in the tibialis anterior (TA) (ankle flexor) and gastrocnemius (Gc) (ankle extensor). An inhalation anesthesia (2%-3% sevoflurane) was used to anesthetize the rats. Stimulation electrodes were inserted while the rats were anesthetized. Rats were secured to a custom-made platform, and electrodes were percutaneously inserted into the TA and Gc on both hindlimbs of the rats.

To insert electrodes near motor points of each muscle, anatomical motor points were referenced, and electrodes were inserted at the point of the greatest contraction after percutaneous stimulation of the target muscle using a low current (frequency, 75 Hz; pulse width, 40 μs; amplitude, 1 mA; duration, 200 ms).

To assess whether stimulation electrodes were positioned at appropriate points near motor points of target muscles, strength-duration (SD) curves were generated for all muscles being studied immediately after the insertion. SD curves show the relationship between the minimum current necessary to establish a stimulation threshold and pulse widths. In this study, the minimum current necessary to cause visible twitching of muscles was plotted against pulse widths of 20, 40, 60, 100, 200, and 500 μs per phase.[12,15,16]

An isolated four-channel stimulator (STG 2004, Multichannel Systems, Cytocentrics) was used. Stimulation parameters were calibrated to those used in previous studies.[12,13] Jung et al. have reported the rapid decrease in the range of motion (ROM) related to muscle fatigue after 15 min of NMES.[13] To investigate the usefulness of kHz frequency stimulation,[17,18] two stimulation patterns (Groups A and B) with different frequencies and amplitudes were used. In Group A (n = 5), the amplitude of the stimulation current was set to 1.5 times the threshold known to produce visible twitches (pulse width, 40 μs; frequency, 75 Hz) obtained from SD curves,[12,13,16] whereas in Group B (n = 5), it was set to three times the threshold (pulse width, 40 μs; frequency, 8 kHz) obtained from SD curves. The interval between stimulations was set at 0.6 s, and the duration of stimulations was 84 ms at TA and 240 ms at Gc. The timing of stimulations of the muscles was calibrated in reference to previous electromyogram data obtained from TM experiments in normal rats.[19] Stimulations were performed for 15 min while the rats were anesthetized.

To evaluate sequential changes in the articular ROM of stimulated ankle joints, we performed 3D kinematic analysis using the KinemaTracer® (Kissei Comtec Co. Ltd.). Color markers were attached to joints of both hindlimbs at the skin surface in the iliac region and in hip, knee, ankle, and MP joints. Four charge-coupled device video cameras were used to film these markers. Kinematic analysis software was used to calculate ankle joint ROM, and sequential changes were evaluated immediately and at 5, 10, and 15 min after stimulation (Fig. 2).

The initial average ROM of each group was 32 ± 9° for Group A and 61 ± 8° for Group B. In both groups, a significant decrease in ROM was observed at 5 min after the onset of stimuli (Group A, $p = 0.001$; Group B, $p = 0.00002$). Compared with Group A, the ROM of Group B was significantly more (p = 0.0006) at all time points measured, and an interaction between stimulation parameters and the period after stimulation ($p = 0.001$) was observed. Figure 6 shows average angles of ankle joints of Group A rats during one stimulation cycle (100%). From ankle joint paths, we confirmed joint movement when walking during the walking stimulation.

Rhythmic stimulation of leg joints using NMES with needle electrodes was successful, but evaluation of sequential ROM using 3D kinematic analysis showed that at 5 min post stimulation, ROM significantly decreased. High-frequency and high-amplitude stimulation was also effective in alleviating decreased ROM due to muscle fatigue. Compared with NMES therapy rodent models that use intramuscular electrodes, this model is less invasive and can be combined with spinal regeneration therapies from the acute stage.

Short-term recovery of interlimb coordination during locomotion in a rodent model with iSCI after NMES therapy[20]

In this study, we tested the hypothesis that NMES movement therapy post iSCI improves intra- and interlimb coordination during locomotion. Ten Fischer rats received an incomplete thoracic spinal cord contusion injury (T9, 150kD Infinite Horizon impactor) and were assigned to one of two groups: those receiving hindlimb movement therapy using NMES (iSCINMES) (n = 5) and those receiving no training (iSCINT) (n = 5); five intact rats served as normal controls. Seven days post recovery, the iSCINMES group rats received NMES therapy via needle electrodes percutaneously inserted into bilateral ankle flexors and extensors for 15 min/d for 3 d. Motor function was evaluated using the Basso, Beattie, Bresnahan (BBB) locomotor scale for first 6 d and again on day 14 post injury. 3D kinematic analysis of TM walking was performed on day 14 post injury.

There was no significant difference for BBB locomotor scale among groups. Rats receiving NMES therapy exhibited improved interlimb coordination. Symmetry indices improved in the iSCINMES group rats.

The efficacy of functional neuromuscular stimulation using kHz to stimulate gait rhythm in rats following SCI[21]

In this study, we examined the effectiveness of NMES using alternating currents in the kHz frequency during gait rhythm stimulation therapy. Tests were performed on 20 mature female Fischer rats. iSCI (T9 level) were made with an Infinite Horizon impactor using a force of 150 kdyn, and NMES was administered for 3 d after day 7 post injury. Needle electrodes were percutaneously inserted near motor points of each muscle in conscious rats, and each muscle in the left and right legs were stimulated for 15 min at two frequencies (75 Hz and 8 kHz) to induce a gait rhythm. Motor function was evaluated using BBB scores and 3D gait analysis. Rats were divided into four groups (five rats/group)—the 75-Hz NMES treatment (iSCINMES 75 Hz), the 8-kHz NMES treatment (iSCINMES 8 kHz), the injury control (iSCI-NT), and the normal (Normal-CT) groups—and compared.

There was no significant difference in the BBB scores among the three groups. Compared with the injury control group in 3D gait analysis, the 8-kHz group showed a significant improvement in the synergistic movement of both hindlimbs. We suggest that kHz stimulation is effective in gait rhythm stimulation using NMES.

Effect of the combination of functional neuromuscular electrical stimulation and TM walking in spinal cord injured rats[22]

The aim of this study was to elucidate the effectiveness of combining NMES therapy and TM training using NMES therapy model.

We used 25 Fischer rats (aged 12 weeks) that were equally divided into five groups: rats with SCI that underwent only NMES therapy, those with SCI that underwent gait training on a TM, those with SCI that underwent both gait training and TM therapies, an SCI control group, and a normal control group. SCI was created by crushing the spine at the T9 level. NMES therapy was initiated 5 d later. The TA and Gc were used for stimulation. NMES was administered to both hindlimbs 15 min/d for 3 consecutive days at a previously defined walking rhythm. The TM group started gait training 15 min/d for 5 consecutive days at 7 d post SCI. The combination group underwent both therapies. Therapeutic effect was evaluated in a 3D gait analysis wherein color markers were placed over each joint of the front and hindlimbs. This was captured using four charge-coupled device cameras while the rats walked on a TM at 14 d post SCI. We found that the interlimb coordination of both hindlimbs, the circular phase, and, in particular, the horizontal movement of the ankle joint improved in the two NMES groups compared with those in the SCI control group. This was interpreted as an indication that their gait pattern was almost normal. An improving trend was also seen in trunk movement during gait in all therapy groups, including the TM group.

In this study, short-term NMES therapy improved locomotor function. Rhythmic NMES, an appropriate load on the hindlimbs, and plantar sensory stimulation may be important in effective rehabilitation.

Future Prospects

Clinically, it is important to plan effective rehabilitation according to the degree of neurological deficit of an individual patient because the state of paralysis is different in each patient. Quantitative assessment of walking function is necessary for effective rehabilitation, and we are now constructing the gait analysis system in collaboration with the rehabilitation division of our hospital (Fig. 1). In future, we will test the effectiveness of various walk training methods using this gait analysis system and will develop an effective rehabilitation support system that will maximize the functional recovery for each patient with lower limb paralysis.

Conclusion

In summary, we successfully developed a less invasive model for NMES therapy that used needle electrodes. Three-dimensional gait analysis revealed improved toe clearance and touchdown phase of both hindlimbs in the NMES group, with a particularly significant improvement in the 8-kHz group. This suggests that stimulation with alternating currents in the kHz frequency is effective in gait rhythm stimulation by NMES. Another

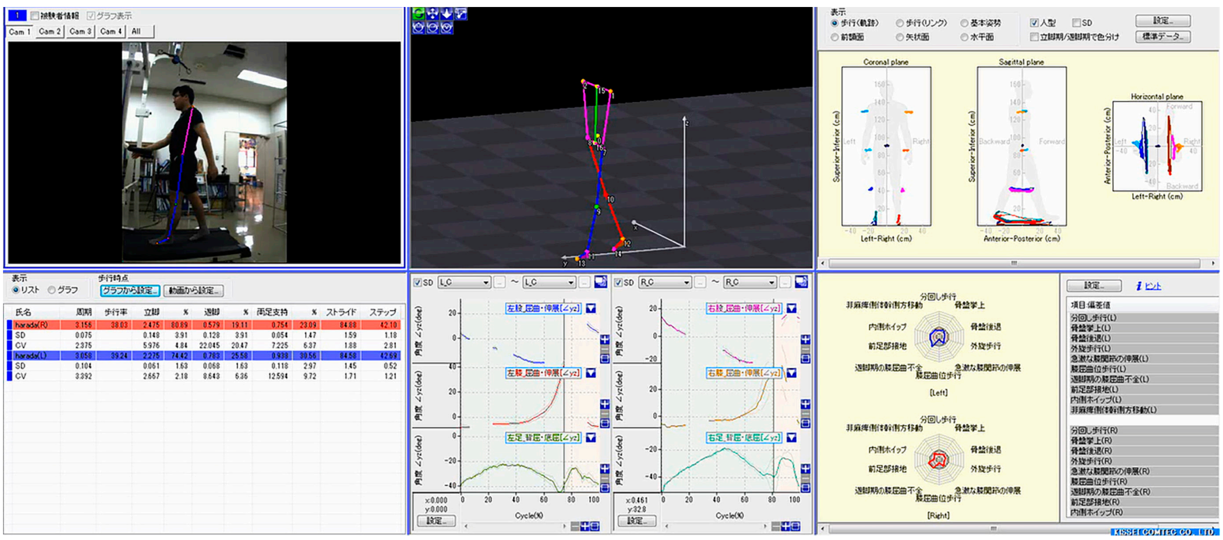

Fig. 1 Three-dimensional gait analysis system.
Camera markers were attached to each joint of the four limbs, and test subject was made to walk at a set speed on the treadmill. The camera markers, which were attached to the lower limbs, were filmed using four charge-coupled device cameras, and the digital data obtained was analyzed and evaluated.

study suggested that more effective locomotor improvement is observed post SCI neurorehabilitation by initiating NMES-supported walking rhythm stimulation therapy in the acute stage. This therapy can then be combined with TM walking or gait training using walking-assist robots once gait training becomes possible after ambulation.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Ramer, L.M., Ramer, M.S.and Steeves, J.D.: Setting the stage for functional repair of spinal cord injuries: a cast of thousands. *Spinal Cord.*, **43(3)**: 134-61,2005.
2. Yara, T., Kato, Y., Kataoka, H., Kanchiku, T., Suzuki, H., Gondo, T., Yoshii, S.and Taguchi, T.: Environmental factors involved in axonal regeneration following spinal cord transaction in rats. *Med. Mol. Morphol.*, **42(3)**: 150-4, 2009.
3. Suzuki, H., Taguchi, T., Kato, Y., Kanchiku, T., Imagama, T., Yara, T., Moriya, A., Muramatsu, K., Tanaka, H.and Gondo, T.: Transplantation of neurospheres derived from bone marrow stromal cells promotes neurological recovery in rats with spinal cord injury. *Med. Mol. Morphol.*, **44(3)**: 131-8, 2011.
4. Yoshida, Y., Kataoka, H., Kanchiku, T., Suzuki, H., Imajo, Y., Kato, H.and Taguchi, T.: Transection method for shortening the rat spine and spinal cord. *Exp. Ther. Med.*, **5(2)**: 384-8, 2013.
5. Murakami, Y., Kanchiku, T., Suzuki, H., Imajo, Y., Yoshida, Y., Nomura, H., Cui, D., Ishikawa, T., Ikeda, E.and Taguchi, T.: Anti-interleukin-6 receptor antibody reduces neuropathic pain following spinal cord injury in mice. *Exp. Ther. Med.*, **6(5)**: 1194-8, 2013.
6. Suzuki, H., Kanchiku, T., Imajo, Y., Yoshida, Y., Nishida, N., Gondo, T., Yoshii, S.and Taguchi, T.: Artificial collagen-filament scaffold promotes axon regeneration and long tract reconstruction in a rat model of spinal cord transection. *Med. Mol. Morphol.*, **48(4)**: 214-24, 2015.
7. Barbeau, H., McCrea, D.A., O'Donovan, M.J., Rossignol, S., Grill, W.M.and Lemay, M.A.: Tapping into spinal circuits to restore motor function. *Brain Res. Rev.*, **30(1)**: 27-51, 1999.
8. Field-Fote, E.C.: Combined use of body weight support, functional electric stimulation, and treadmill training to improve walking ability in individuals with chronic incomplete spinal cord injury. *Arch. Phys. Med. Rehabil.*, **82(6)**: 818-24, 2001.
9. Wirz, M., Zemon, D.H., Rupp, R., Scheel, A., Colombo, G., Dietz, V. and Hornby, T.G.: Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord injury: a multicenter trial. *Arch. Phys. Med. Rehabil.*, **86(4)**: 672-80, 2005.
10. Ring, H.and Rosenthal, N.: Controlled study of neuroprosthetic functional electrical stimulation in sub-acute post-stroke rehabilitation. *J. Rehabil. Med.*, **37(1)**: 32-6, 2005.
11. Jung, R., Ichihara, K., Venkatasubramanian, G.and Abbas, J.J.: Chronic neuromuscular electrical stimulation of paralyzed hindlimbs in a rodent model. *J. Neurosci. Methods.*, **183(2)**: 241-54, 2009.
12. Ichihara, K., Venkatasubramanian, G., Abbas, J.J.and Jung, R.: Neuromuscular electrical stimulation of the hindlimb muscles for movement therapy in a rodent model. *J. Neurosci. Methods.*, **176(2)**: 213-24, 2009.
13. Jung, R., Belanger, A., Kanchiku, T., Fairchild, M. and Abbas, J.J.: Neuromuscular stimulation therapy after incomplete spinal cord injury promotes recovery of interlimb coordination during locomotion. *J. Neural. Eng.*, **6(5)**: 055010. doi:10.1088/1741-2560/6/5/055010.Epub., 2009.
14. Kanchiku, T., Kato, Y., Suzuki, H., Imajo, Y., Yoshida, Y., Moriya, A., Taguchi, T.and Jung, R.: Development of less invasive neuromuscular electrical stimulation model for motor therapy in rodents. *J. Spinal Cord Med.*, **35(3)**: 162-9,2012.
15. Crago, P.E., Peckham, P.H., Mortimer, J.T.and Van der Meulen, J.P.: The choice of pulse duration for chronic electrical

stimulation via surface, nerve, and intramuscular electrodes. *Ann. Biomed. Eng.*, **2(3)**: 252-64, 1974.

16. Kanchiku, T., Lynskey, J.V., Protas, D., Abbas, J.J.and Jung, R.: Neuromuscular electrical stimulation induced forelimb movement in a rodent model. *J. Neurosci. Methods.*, **167(2)**: 317-26, 2008.
17. Ward, A.R.: Electrical stimulation using kilohertz frequency alternating current. *Phys. Ther.*, **89(2)**: 181-190, 2009.
18. Ward, A.R.and Robertson, V.J.: The variation in fatigue rate with frequency using kHz frequency alternating current. *Med. Eng. Phys.*, **22(9)**: 637-46, 2000.
19. Thota, A.K., Watson, S.C., Knapp, E., Thompson, B.and Jung, R.: Neuromechanical control of locomotion in the rat. *J. Neurotrauma.*, **22(4)**: 442-65, 2005.
20. Moriya, A., Kanchiku, T., Imajo, Y., Yoshida, Y., Suetomi, Y., Nishida, N., Takahashi, Y.and Taguchi, T.: Short-term recovery of interlimb coordination during locomotion in a rodent model with incomplete spinal cord injury after minimally-invasive neuromuscular electrical stimulation therapy. *Exp. Ther. Med.*, In press, 2017.
21. Kanchiku, T., Suzuki, H., Imajo, Y., Yoshida, Y., Moriya, A., Suetomi, Y., Nishida, N., Takahashi, Y.and Taguchi, T.: The efficacy of neuromuscular electrical stimulation with alternating currents in the kilohertz frequency to stimulate gait rhythm in rats following spinal cord injury. *Biomed. Eng. Online.*, 14:98. doi: 10.1186/s12938-015-0094-5, 2015.
22. Takahashi, Y., Kanchiku, T., Suzuki, H., Imajo, Y., Yoshida, Y., Moriya, A., Suetomi, Y., Nishida, N.and Taguchi, T.: Effect of the combination of functional neuromuscular electrical stimulation and treadmill walking in spinal cord-injured rats. *Exp. Ther. Med.*, In press, 2017.