Effects of TENS and EA on the Expression of NT-3 on Lumbar Spinal Cord after Crush Injury of Extensor Digitorum Muscle

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Purpose: The purpose of this study to investigate the effect of TENS and electroacupuncture (EA) on injured muscle recovery and to compare the difference between TENS and EA. Methods: 18 S-D rats were produced surgically for crush injury on Extensor digitorum. TENS and EA stimulation were applied on acu-point that Zusanli (ST36) and Taijun (Liv3) of each hindlimb as approximitely 2Hz for 15 minutes. The rats were sacrificed on 1, 3, 7 post operation day, and the spinal level was immunohistochmical stainied. Results: The expression of NT-3 on the spinal level related with injured muscle between Ex-1 group and Ex-2, there is no significant difference. But between Con and Ex-1/Ex-2, there was little difference. In the same indivudual, we confirmed more NT-3 expression on the same side of injured muscle than on another side of gray matter in posterior horn. Conclusion: There is no difference, which is more effective to recover of injured muscle, between EA and TENS on injured muscle. *(J Kor Soc Phys Ther 2006;18(2):59-64)*

Key Words : Muscle crush injury, NT-3, EA, TENS

I. Introduction

Skeletal muscle contusion occur frequently in sports. Such injuries are typically the result of a blunt, non-penetrating impact between muscle and rigid object (Crisco et al., 1996). In Oriental medicine, acupuncture has been employed as a treatment of many disorders. Zusanli (ST 36) and Taijun (Liv 3) are one of the most effective acupuncture points, with a wide range of effects such as analgesic, spasmolytic and homeostatic (Hwang et al., 2001), especially for treatment of low extremity (Hyodo, 1976).

Electroacupuncture (EA) has long been used to relieve pain. In clinic, EA is an established adjuvant analgesic modality for the treatment of muscle injury. It has been report that EA had potent analgesic effect in muscle injury on rat model (Kim et al., 2002). Empirically, Zusanli and Taijun are the most favorable for the treatment on lower extrimity injuries and disorders such as muscle injury, neuropathic disorders and even fracture (Hyodo, 1976).

The neurotrophins brain-drived neuro-trophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), are expressed in embryonic and postnatal muscles where they function as neurotrophic factors for the α and β - motor neurons and the primary afferents innervating the muscle spindles and golgi organs (Copray and Brouwer, 1997). NT-3, a member of the neurotrophin family, appeared

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an interesting candidate: it is naturally produced by skeletal muscle both prenatally and postnatally. It appear on adult spinal motor neurons (Haase et al., 1998).

The purpose of this study is comaprison between the effects of EA and TENS on the expression of neurotrophins at the spinal cord after muscle injury. We suggest which intervention is more effective for recover of muscle injury.

II. Material and Methods

1. Animal groups

Eighteen Sprague-Dawley rats which are weighting between 250g and 300g were used. All rats were divided into three groups as TENS group (n=3), EA group (n=3) and control group (Con; n=3). The rats were sacrificed a rat on 1, 3, 7 day after operation.

2. Surgical procedures

The rats, after shaving hindlimb, were anethetized with ketamin HCL (Yuhan, Korea) and Xylazine hydrocholride (Byel-korea, Korea) (1:1; 2ml/kg). Skin and muscle facia were opened for exposing extensor digitorum between peronius and peroneus longus. Extensor digitorum was pinched by homostatic forceps for 30 seconds. At the last, incision wound were sutured every 5㎜ and disinfected by mercurochrome.

3. EA and TENS

For EA stimulation, two stainless needles were inserted into acu-points Zusanli and Taijun of each hindlimb (Figure 1). The two needles were connected with the output terminals of stimulator (Rebirth-102, Saelk Medical Co. Ltd., Korea) that delivered symmetrical biphasic waves at a frequency of 2㎐. The intensity of EA was applied at approximately 1㎃ (increasing intensity until we can observe contraction of muscle) for 15 minutes.

For TENS, two electrodes (2mm×2mm) were attached on the same place with EA. For stimulation, continuous waves at a frequency of 2㎐. The intensity of EA and TENS stimulation was determined to be the minimum current to induce moderate muscle contraction.

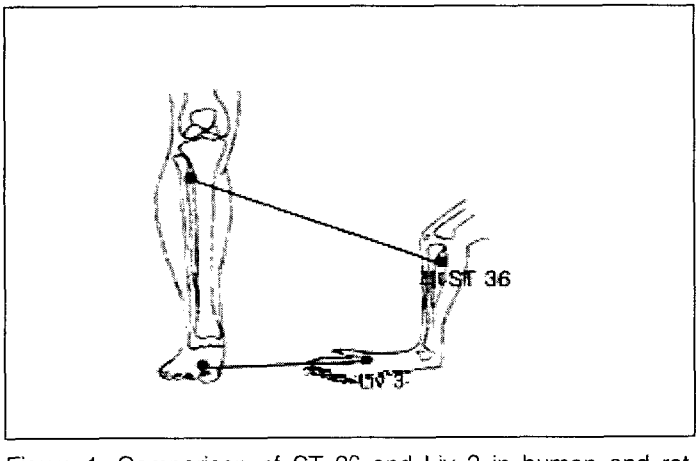

Figure 1. Comparison of ST 36 and Liv 3 in human and rat.

4. Immunohistochemistry

The animals were anethetized with intraperitonial injection of ketamin HCL (Yuhan Corp., Korea) and Xylazine hydrocholride (Bayer-Korea Corp., Korea) (1:1; 2ml/kg) and sacrificed via inter cardiac perfusion with 0.9% NaCl, followed by 4% para-formaldehyde fixation. The spinal cord was removed and postfixed for 2 hours in the same fixative at room temperature, then 25% sucrose solution for 24 hours. Tissues were cut on the cryostat at 30㎛ at L5-S1. the sections were immersed in 0.01M, Ph7.6, phosphate-buffer(PB) for free-floating staining.

The sections were incubated 24 hours at 4°C with an antibody against NT-3. After that, these were rinsed with 0.01M PB for 30 minutes three times. The sections were incubated with primary antibody (mouse monoclonal anti-neurotrophin-3, Chemicon, USA, 1:200) for 24 hours at room temperature. After this process, these were rinsed by 0.01M PB for 30 minutes three times, and the secondary anti-body (anti-mouse IgG, Vector Laboratories Inc., USA, 1:25) was incubated for 90 minutes at room temperature.

After this process, these were rinsed by 0.01M PB for 30 minutes three times, the sections were incubated the tertiary anti-body ABC-kit (Vector Labratoried Inc. USA) for 60 min. After rinsed by 0.01M PB for 30 minutes three times, making DAB reaction for 10 minutes. Risened by 0.01M PB, the sections were then mounted, dehydrated and covered. We observed with optical microscope (Olympus BX50, Olympus, Japan) for confirming expression of NT-3.

III. Results

As the result of the experiment, the expression of NT-3 on the spinal level related with injured muscle between Ex-1 group and Ex-2, there is no significant difference. But between Con and Ex-1/ Ex-2, there was little difference(Table 1, Figure 2).

In the same indivudual, we confirmed more NT-3 expression on the same side of injured muscle than on another side of gray matter in posterior horn.

'+' is the smallest expression of NT-3 at the spinal cord

Table 1. The changes of NT-3 Expression in 1, 3, 7 days on each Group

		1 day	3 day	7 day
NT-3 expression	Control Group	++	++	+
	TENS Group	+++	++	++
	EA Group	+++	++	++

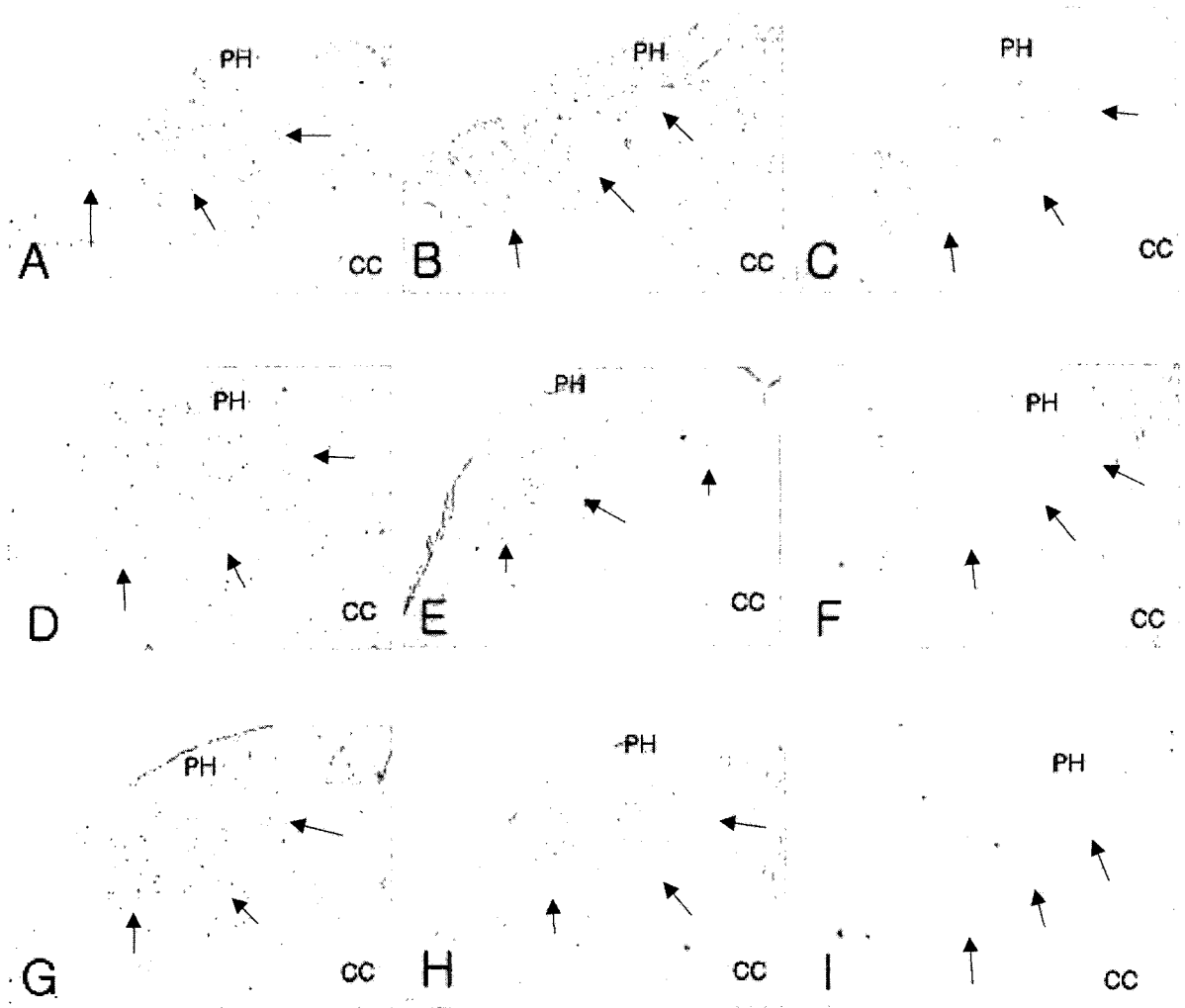

Figure 2. The expression of NT-3 in spinal cord posterior horn by electroacupuncture (EA) on extensor digitorum longus muscle induced crash injury. A, B, C; Control Group (×40). D, E, F; TENS Group (×40). G, H, I; EA Group (×40). A, D, G; Sacrificed 1 day of injury. B, E, H; Sacrificed 3 days of injury. C, F, I; Sacrificed 7 days of injury. CC=central canal of spinal cord, PH=posterior horn of spinal cord.

Ⅳ. Discussion

As a result of this study, on the spinal cord level which related with injured muscle, we could observe expression of NT-3. Especially at 1st day, There was difference between control and TENS/EA group, But we compared TENS with EA groups, there was no difference of NT-3 expression on posterior horn of lumbar spinal cords, also at 3rd day group and 7th day group. It means TENS group and EA group are effective on muscle injury related with reconnection of motor neurons (Greenhalgh, 1996; Kurek et al., 1997).

Acupuncture is an ancient Chinese method to treat disease, injury and relieve pain. Some prior studies showed that EA was more effective than manual acupuncture. For example, a 70% rate of success for pain elimination or modulation has been reported clinically in patients with low back strain, arthritis, myofascial discomfort and other painful disorders (George et al., 1998).

The major function of neurotrophic factors present in spinal cord was thought to be related to the maintenance and plasticity of neuromuscular connection at injured muscle; as such, they could play a role in the regenerative response at the neuromuscular junction after denervation. The neurotrophins BDNF, NT-3 and NT-4 have demonstrated similar effects (Lewin and Barde, 1996; Heather et al., 2005). The roles are; (1) found in developing skeletal muscle during naturally occuring motor neuron cell death, (2) retrogradely transported by motor neurons and (3) observed to be imported for spinal motor neuron survival in different experimental approaches (Yan et al., 1992; DiStefano et al., 1992; Sendtner et al., 1992; Koliatos et al., 1993, 1994; Henderson et al., 1993). This muscle derived NT-3 may acts an instructive feedback messenger for innervating I a afferents and γ-motoneurons and/or as a stabilizing factor, essential for the maintenance of a functional neuromuscular connection. It had to be established whether the level of expression of NT-3 is higher or not in spinal cord.

Prior studies suggested low frequency (2~5Hz) and high frequency (100hz) for pain control with EA and TENS (Cheng et al., 1981; Pert et al., 1981; Pomeranz et al., 1977). Ironically there are different opinion about the intensity and frequency of stimulation. But, generally, high intensity which is strong enough stimulation for effect to Aδ and C nerve fibers is more effective and longer lasting.

In this study, we used continuous modes at a frequency of 2Hz. 2Hz frequency was most effective for expression of enkephalins and dynorphins biological functions in the CNS (Wang et al., 1992; Well et al., 1995).

V. Conclusion

The conclusions from this study and be restated as follow; (1) EA and TENS is effective for expression of NT-3 on spinal cord. (2) TENS is as effective as EA for expression of NT-3 on spinal cord. Simply, there is no difference, which is more effective to recover of injured muscle, between EA and TENS on injured muscle.

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전침자극과 경피신경전기자극이 장지신근의 압좌손상 후 척수수준에서의 NT-3 발현에 미치는 영향

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<국문초록>

목적: 전침과 경피신경전기자극이 근육 손상에 미치는 차이와 효과에 대하여 알아보기 위하여 이 실험을 시행하였다. 방법: 8-10주령의 건강한 S-D계 흰쥐 18마리(250-300g)가 이 실험에 사용 되었으며 각 실험은 수술을 통하여 장지신근을 30초간 수술용 겸좌로 압좌한 후 무작위로 세 그룹으로 나누었다. 아무런 처치를 하지 않은 그룹을 대조군(Con), TENS 적용 그룹을 실험군-1(Ex-1), 전침 적용군을 실험군-2(Ex-2)으로 정하여 전기 자극을 빈도 2Hz, 강도 1mA, 통전시간 15분 씩 7일간 적용한 후 각 그룹에서 2마리씩 1, 3, 7일에 희생하여 척수의 요수부위를 적출하여 이를 NT-3 항체 처리하여 염색하였다. 결과: 실험군-1, 2는 대조군에 비하여 조금 더 많은 NT-3가 발현됨을 관찰 할 수 있었지만. 각 실험군 간의 큰 차이는 발견하지 못하였다. 결론: TENS와 전침적용의 분자수준에서의 큰 차이점은 없다고 사료된다.

핵심단어: 압좌손상, NT-3, 전침자극, 경피신경전기자극