한국심리학회지 : 생물 및 생리
Korean Journal of Biological and Physiological Psychology
2002. Vol. 14, No. 1, pp. 39-46

# Facilitatory Effects of Acidic Fibroblast Growth Factor on Functional Recovery after Sciatic Nerve Injury*

Bae Hwan Lee Eun Jung Kim Kyung Hee Lee Se Jung Jung Yong Gou Park†

Medical Research Center, Brain Research Institute, Department of Neurosurgery,
Yonsei University College of Medicine

Acidic fibroblast growth factor(aFGF) has been shown to increase regeneration across a gap between the proximal and distal stumps of a transected nerve. In the present study, we examined sensory axon regeneration using an electrophysiological technique in order to investigate the effects of aFGF on regeneration of peripheral nerve axons after injury. Under pentobarbital anesthesia, male Sprague-Dawley rats were subjected to one of three different types of injury(crush, ligation, and sham injury models). The rats received i.v. injections of aFGF for ten days from the day of surgery. Twenty five days after injury, the rats were anaesthetized with urethane and the somatosensory evoked potentials(SSEPs) were recorded following sciatic nerve stimulation. aFGF significantly shortened the latencies of the SSEPs in crush injury model compared to the vehicle treated rats. In the case of ligation injury model, aFGF tended to shorten the latencies but the differences between aFGF and vehicle treated rats were not statistically significant. These results suggest that aFGF facilitates functional recovery after sciatic nerve injury.

* This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republicf of Korea. (HMP-98-NM-2-0034)

† Corresponding author : Yong Gou Park / Department of Neurosurgery, Yonsei University College of Medicine, C.P.O.. Box 8044, Seoul 120-752, Korea / ygpark@yumc.yonsei.ac.kr

Injury to a peripheral nerve often results in functional disorders in humans and animals. In this case, physiologic repair is important for functional recovery or regeneration following nerve injury and the presence of specific neuronotropic factors might enhance the process.

Acidic fibroblast growth factor(aFGF) has been shown to be present in high concentrations in peripheral nerves compared to other parts of the nervous system(Eckenstein, Shipley, & Nishi, 1991). In the intact sciatic nerve of the rat, the levels of aFGF are about 1000 fold higher than those of nerve growth factor(Ishikawa, Nishikori, Furukawa, Hayashi, & Furukawa, 1992).

Endogenous aFGF has been shown to be present in both motor and sensory components of peripheral nerves(Elde, Cao, Cintra, Brelje, Pelto-Huikko, Junttila, Fuxe, Pettersson, & Hokfelt, 1991; Ishikawa et al., 1992) but different factors may regulate functional recovery in these two populations.

In the present study, we examined the functional recovery of sensory axons using an electrophysiological technique in order to investigate the effects of aFGF on conduction of sensory nerve axons after injury.

## MATERIALS AND METHODS

### Subjects and Surgery

A total of thirty five male Sprague-Dawley rats(Daehan Biolink, Korea) with body weights between 250-350g were used. Anaesthesia was induced by intraperitoneal injection of sodium pentobarbital(Entobar®). The left sciatic nerve was exposed where it emerged from beneath the middle gluteal muscle.

Each rat received one of three different types of injury. For example, in the case of crush (crush group), the nerve was crushed at this marked point using a pair of forceps with 0.5-mm-wide tips. In ligation group, the nerve was tightly ligated with 4.0 silk thread. In sham group, the surgery was identical except for leaving the nerve intact. The wound was closed in layers, and the rat allowed to recover from anaesthesia. During recovery the rats were observed continuously until the righting reflex was restored and then returned to their home cage, where they were group housed.

Rats received i.v. injections of aFGF with a dose volume of 1 ml/kg or vehicle into the tail vein daily for ten days, starting on day 0(day of surgery).

### Electrophysiological Recording

Twenty five days after injury, the rats were anaesthetized with urethane(1.25g/kg). The trachea, left jugular vein and left carotid artery were cannulated to enable artifical respiration, the administration of drugs and solutions and the recording of systemic arterial blood pressure,

respectively. Body temperature was maintained at 37.5℃ by an electric heating blanket with feedback control from a rectal probe thermistor. The injured nerve was exposed and freed from surrounding connective tissue along its entire length from above the site of the injury to below the trifurcation at the level of the knee. Once the preparative surgery was completed, the rat was given pancuronium bromide(2mg/kg/h) to prevent movement artifacts and artificially ventilated. Adequate anaesthesia was ensured in the absence of neuromuscular block by maintaining areflexia. After neuromuscular blockade the depth of anaesthesia was monitored by observing pupillary reflexes and maintained such that no precipitate changes in blood pressure were observed on the application of a noxious pinch stimulus.

The sciatic nerve was placed on silver bipolar stimulating electrodes and stimulated(0.1ms square pulse) with increasing intensity ranging from 0.05 to 9 mA. The somatosensory evoked potentials (SSEPs) were recorded from the cerebral cortex using disk-type bipolar electrode. As 6 mA square pulses tended to produced consistent SSEPs, data from SSEPs evoked by 6 mA current were analyzed. For each measurement, the responses to 20 stimuli were collected and averaged using a CED 1401 interface and a 586 personal computer.

## Statistical Analysis

Statistical analysis was performed on the raw data from SSEPs using the SigmaStat software package. Differences between groups(aFGF vs. vehicle) in each injury type were tested with Student's t-test. Differences for which $p<0.05$ were taken to be statistically significant.

## RESULTS

At the hindlimb area of the cortex, SSEPs were recorded on stimulation of the sciatic nerve. Results of the electrophysiological studies are summarized in the figures. At 25 days, the latencies of the somatosensory evoked potentials (SSEPs) in the aFGF treated rats tended to be substantially shortened than those of the vehicle treated rats, indicating functional recovery from nerve injury. Figure 1 shows representative wave forms of SSEPs evoked by stimulation of the sciatic nerve in each group with different types of nerve injury. The amplitudes of the SSEPs tended to be higher in the aFGF treated rats than those of the vehicle treated rats. However, as the trend of changes in the amplitudes were not consistent, the data of amplitudes were not included in statistical analysis. In contrast, the latencies of SSEPs were consistent and analysed statistically.

In figures 2, the mean latencies of the SSEPs evoked by sciatic stimulation with 0.4 mA square pulses were compared. In sham operated rats, the latencies of the SSEPs of aFGF injected rats

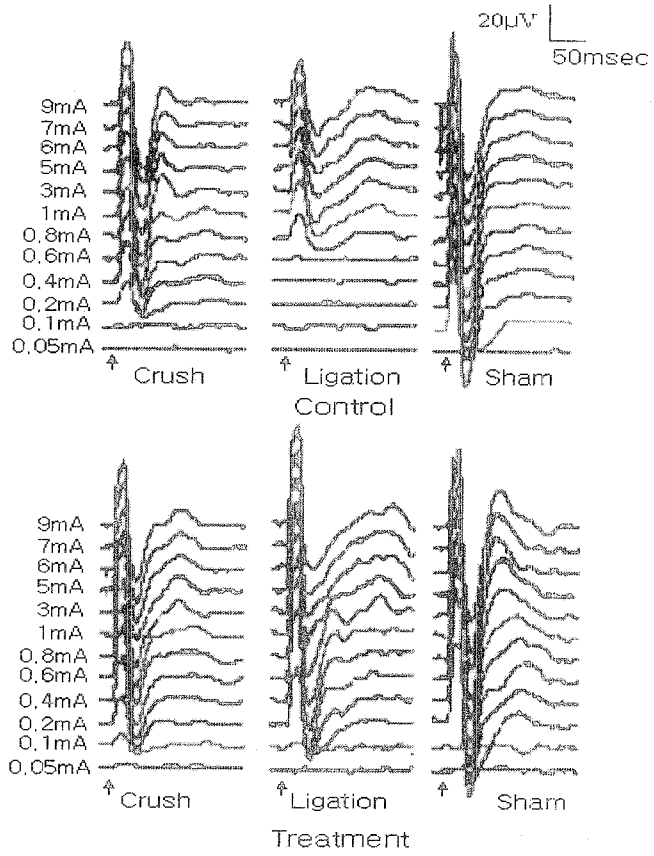


Figure 1. Representative wave forms of somatosensory evoked potentials from each group on day 25 p.o. Arrow indicates stimulus artifact.

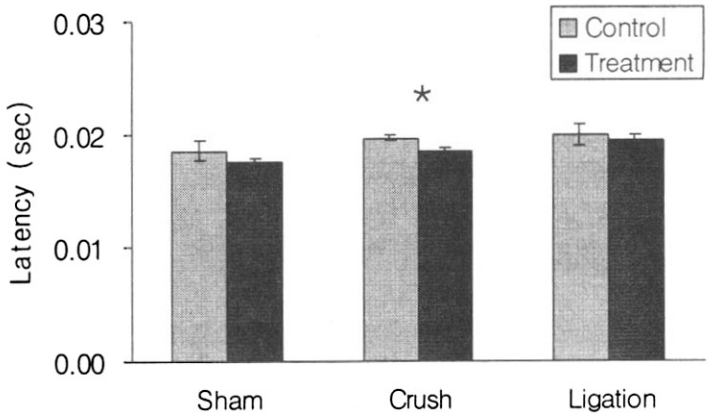


Figure 2. Comparisons of mean latencies of the somatosensory evoked potentials following sciatic nerve stimulation on p.o. 25 day. (*p<0.05).

(n=6) were similar to those of vehicle treated rats(6) ($t(10)=1.10$, $p>0.05$). In crush injury model, the latencies of the SSEPs of aFGF injected rats(n=6) were shortened significantly compared to those of the vehicle treated rats (n=6) ($t(10)=3.19$, $p<0.05$). In the case of ligation injury model, the latencies of the SSEPs of aFGF injected rats(n=6) were not different from those of the vehicle treated rats(n=5) ($t(9)=0.46$, $p>0.05$).

## DISCUSSION

According to an immunocytochemical study, high levels of aFGF are naturally found in the cell bodies of neurons whose axons run in peripheral nerves(Elde et al., 1991). This endogenous growth factor may play a role in physiologic repair after nerve injury.

In the present study, we tested the effects of exogenously applied aFGF on regeneration following a crush or ligation injury of the sciatic nerve in the rat. The administration of aFGF systemically once daily significantly enhanced the regeneration of sensory axons in the sciatic nerve of the rat after a crush injury, whereas there were no effects in the other injury model. This phenomenon is not easy to explain. Two hypothesis may be helpful to explain it as follows:

Firstly, aFGF may act on neuronal cell directly. A crush injury causes only very local damage to the non-neuronal supporting cells and does not disrupt the endoneurial tubes. In the absence of a gap, any enhancement of functional recovery by aFGF is likely to be attributable to increased axonal extension, rather than due to an indirect effect mediated by enhancing the outgrowth of supporting cells. Therefore, aFGF may exert its effects directly on neuronal cells. It has been shown to promote the survival, neurotransmitter synthesis and neurite outgrowth of neuronal cells in culture independently of its actions on non-neuronal cells(Unsicker, Grothe, Ludecke, Otto, & Westermann, 1993). Non-specific fibroblast growth factor receptor mRNA has been reported to be present in motoneurons(Wanaka, Johnson, & Millbrandt, 1990), although there is little information on the presence of fibroblast growth factor receptors on primary sensory afferents. It is also possible that fibroblast growth factor receptors appear on neurons *de novo* after injury.

Secondly, aFGF may also have indirect effects on neuronal cells, via an action on the non-neuronal supporting cells in peripheral nerve (Unsicker et al., 1993). aFGF immunoreactivity in peripheral nerve is found associated with the plasma membrane of axons on the cytoplasmic side(Elde et al., 1991). Disruption of the axon membranes during Wallerian degeneration is likely to release endogenous aFGF, which may act as the first signal initiating the cellular and

molecular cascade necessary to initiate and sustain regeneration before being fully degraded or diffusing away(Eckenstein et al., 1991). For example, both isolated axonal membranes and aFGF have strong mitogenic effects on Schwann cells in vitro. Thus, the release of endogenous aFGF from the damaged axons may promote the massive increase in Schwann cells in an injured nerve(Raivich & Kreutzberg, 1993).

It seems to be possible that aFGF exerts its effects on neuronal cell either directly or indirectly. However, the precise mechanisms that aFGF enhances functional recovery after injury is uncertain. Here, we can postulate two possibilities. Firstly, aFGF prevents post-axotomy neuronal death peripheral nerve(Cuevas, Carceller, & Gimenez-Gallego, 1995a). There is also evidence that aFGF prevents death of spinal cord motoneruons in newborn rats after nerve section (Cuevas, Carceller, & Gimenez-Gallego, 1995b). Secondly, aFGF also enhances peripheral nerve regeneration in vivo(Cordeiro, Seckel, Lipton, D'Amore, Wagner, & Madison, 1989). This kind of progressive regeneration may contribute to the gradual functional recovery.

The present study demonstrated that a stimulatory effect on functional recovery could be obtained with systemic daily administration of aFGF. This indicates that aFGF may be clinically useful when given locally to promote nerve repair and also when given systemically to treat patients with mono- or polyneuropathy.

## REFERENCES

Bisby, M. A. & Chen, S. (1990). Delayed Wallerian degeneration in sciatic nerves of C57BL/Ola mice is associated with impaired regeneration of sensory axons. *Brain Research*, 530, 117-120.

Cordeiro, P. G., Seckel, B. R., Lipton, S. A., D'Amore, P. A., Wanger, J., & Madison, R. (1989). Acidic fibroblast growth factor enhances peripheral nerve regeneration *in vivo*. *Plastic and Reconstructive Surgery*, 83, 1013-1019.

Cuevas, P. C., Carceller, F., & Gimenez-Gallego, G. (1995a). Acidic fibroblast growth factor prevents post-axotomy neuronal death of the newborn rat facial nerve. *Neuroscience Letters*, 197, 183-186.

Cuevas, P. C., Carceller, F., & Gimenez-Gallego, G. (1995b). Acidic fibroblast growth factor prevents death of spinal cord motoneruons in newborn rats after nerve section. *Neurological Research*, 17, 396-399.

Cordeiro, P. G., Seckel, B. R., Lipton, S. A., D'Amore, P.A., Wagner, J., & Madison, R. (1989). Acidic fibroblast growth factor enhances peripheral nerve regeneration in vivo. *Plastic and Reconstructive Surgery*, 83, 1013-1019.

Eckenstein, F. P., Shipley, G. D., & Nishi, R. (1991). Acidic and basic fibroblast growth factors in the nervous system: distribution

and differential alteration of levels after injury of central versus peripheral nerve. *Journal of Neuroscience*, 11, 412-419.

Elde, R., Cao, Y., Cintra, A., Brelje, T. C., Pelto-Huikko, M., Junttila, T., Fuxe, K., Pettersson, R. F., & Hokfelt, T. (1991). Prominent expression of acidic fibroblast growth factor in motor and sensory neurones. *Neuron*, 7, 349-364.

Ishikawa, R., Nishikori, K., Furukawa, Y., Hayashi, K., & Furukawa, S. (1992). Injury-induced reduction of acidic fibroblast growth factor levels in the distal parts of the rat sciatic nerve. *Neuroscience Letters*, 135, 113-116.

Laird, J. M. A. & Bennett, G. J. (1991). Dorsal root potentials and afferent input into the spinal cord in rats with an experimental peripheral neuropathy. *Brain Research*, 584, 181-190.

Lunn, E. R., Petty, V. H., Brown, M. C., Rosen, H., & Gordon, S. (1989). Absence of Wallerian degeneration does not hinder regeneration in peripheral nerve. *European Journal of Neuroscience*, 1, 27-33.

Politis, M. J. (1986). Retina-derived growth-promoting extract supports axonal regeneration *in vivo*. *Brain Beseerch*, 364, 369-371.

Raivich, G. & Kreutzberg, G. W. (1993). Peripheral nerve regeneration: role of growth factors. *International Journal of Developmental Neuroscience*, 11, 311-324.

Unsicker, K., Grothe, C., Ludecke, G., Otto, D., & Westermann, R. (1993). Fibroblast growth factors: their roles in the central and peripheral nervous system. In S.E. Louglin and J. H. Fallon(Eds.), Neurotropic Factors(pp. 313-338). New York: Academic Press.

Wall, P. D. & Devor, M. (1981). The effect of peripheral nerve injury on dorsal root potentials and on transmission of afferent signals. *Brain Research*, 209, 95-111.

Walter, M. A., Kurouglu, R., Caulfield, J. B., Vasconez, L. O., & Thompson, J. A. (1993). Enhanced peripheral nerve regeneration by acidic fibroblast growth factor. *Lymphokine Cytokine Research*, 12, 135-141.

Wanaka, A., Johnson, E. M., & Millbrandt, J. (1990). Localisation of FGF receptor mRNA in the adult rat central nervous system by in situ hybridisation. *Neuron*, 5, 267-281.

한국심리학회지 : 생물 및 생리
Korean Journal of Biological and Physiological Psychology
2002. Vol. 14, No. 1, pp. 39-46

# 좌골신경 손상 후 기능회복에 미치는 acidic fibroblast growth factor의 효과

이 배 환 김 은 정 이 경 희 정 세 정 박 용 구
연세의대 임상의학연구센터, 뇌연구소, 신경외과학교실

Acidic fibroblast growth factor(aFGF)는 절단된 신경의 간격을 건너 일어나는 재생을 증진시키는 것으로 알려져 있다. 본 연구에서 저자들은 신경손상후 말초신경 축삭의 재생에 미치는 aFGF의 효과를 알아보기 위해 전기생리학적 기법을 이용하여 기능회복 정도를 평가해 보고자 하였다. pentobarbital 마취 하에서, 수컷 흰쥐의 좌골신경에 압착(crush), 결찰(ligation), 모의(sham) 손상 등 세가지 유형의 손상을 발생시켰다. 쥐들에게 수술날짜를 포함하여 10일 동안 aFGF를 정맥주입하였다. 신경손상후 25일째에 쥐들을 urethane으로 다시 마취하고, 좌골신경의 자극에 따른 체감각 유발전위(SSEP)를 기록하였다. aFGF는 압착 손상 모델에서 용매를 처치한 집단에 비해 SSEP의 잠재기를 유의미하게 단축시켰다. 결찰모델의 경우, aFGF는 SSEP의 잠재기를 단축시키는 경향이 있었지만, aFGF와 용매를 처치한 동물들간의 차는 통계적으로 유의미하지 않았다. 이러한 결과는 aFGF가 좌골신경 손항 후 기능회복을 촉진시킨다는 것을 시사하는 것이다.