AN EFFECTIVE AND SAFE METHOD OF OPIOID GENE-TRANSFER INTO THE CENTRAL NERVOUS SYSTEM FOR NEUROPATHIC PAIN

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**Introduction:** Several kinds of abnormal sensations such as allodynia, hyperalgesia, and continuous spontaneous pain are developed after peripheral nerve injuries. These abnormal sensations are often unresponsive to conventional treatments. Therapeutic gene transfer is emerging as an attractive method for the development of novel treatments for pain. The aim of the present study is development of safe and effective gene transfer method to deliver opioids continuously to CNS. In the present study, we performed an ex vivo gene transfer with non-viral vector including proenkephaline gene to autologous macrophages, then the cells were transplanted to the CNS by intrathecal injection.

**Materials and Methods:** 1) Chronic constriction injury of sciatic nerve model (CCI model).

Male Wistar rats (350 g-weight, purchased from Japan Clea Co., Japan) were used in this study. Under general anesthesia by halothane, the rat sciatic nerve was carefully exposed. CCI was produced in the left side by ligation using polyglucolic acid strings (4-0, Nescocompany, Osaka, Japan) at four points. The polyglucolic acid expands with absorption of moisture after placing in the animals. Therefore, the constriction slowly tighten after the operation. Usually, stable hypersensitivities such as allodynia and hyperalgesia was observed three days after the CCI. The research protocol was accepted by the ethical committee for animal experiments of Ehime University (Ehime, Japan).

2) Collection of autologous macrophages and proenkephalin gene transfer.

When the animals successively revealed allodynia and hyperalgesia at 72h after the CCI, intraperitoneal autologous macrophages were collected and ex vivo gene transfer by electroporation was performed. Intraperitoneal space was rinsed with 30 ml of Dulbecco’s modified minimal essential medium (DMEM) via midline incision of abdomen. The collected DMEM contained numerous amounts of macrophages. The proenkephalin gene was transferred into the macrophages by electroporation. The proenkephalin gene-contained plasmid (0.1 μg/μl: pEGFPLuc Vector, Clontech Inc. U.S.A.) was mixed with 200 μl of the cells (1,000,000cells /ml). Then, six electric pulses of 20 V, 20 ms were applied by electroporator (CUY 21, NEPA GENE Co., Japan). 100 μl of the gene incorporated macrophages (100,000cells) was injected into subarachnoid space at the 4-5th intervertebral level. In the vehicle animals, pEGFPLuc Vector without proenkephalin gene was used instead of the proenkephalin gene-contained plasmid.

3) Evaluation of thermal hyperalgesia.

To evaluate the withdrawal threshold to paw thermal stimulation, we used the Hargreaves’ plantar test apparatus (Ugo Basile, Varese, Italy). Rats were placed on a 2 mm thick glass floor, then a mobile infrared heat generator with an aperture of 10 mm in diameter was aimed at the rat’s hind paw under the floor. When the rats felt pain and withdrew their paw, the power shut off and the reaction time (the withdrawal latency of the paw) was recorded automatically. The temperature of the glass floor was kept at 22.5-23.5°C.

4) Evaluation of allodynia.

The rats were placed on a wire-mesh platform. A graded series of logarithmic von Frey filaments were applied perpendicularly to the plantar surface of hind paw. Sequentially increasing and decreasing the stimulus strength (up-down method) determined the threshold to withdraw.

**Results:** In the animals undergone the CCI treatment, apparent hypersensitivities were observed at 72 hours after the CCI. The intrathecal transplantation of the macrophage modified by gene transfer ex vivo to secrete proenkephalin was done after the sensory evaluation at 72 hours after the ligation. In the withdrawal latency for heat stimulation test (a), animals showed decrease of withdrawal threshold (heat hyperalgesia) at 72 hours after the ligation. Seven days after the gene transfer, the withdrawal threshold to heat stimulation returned to the normal level (pre-CCI). The effect of gene transfer was continued at least 6 weeks after the transplantation. On the other hand, no effect on hyperalgesia was observed in the vehicle animals. In the withdrawal latency for mechanical stimulation test (b), animals showed decrease of withdrawal threshold (allodynia) at 72 hours after the ligation. Seven days after the gene transfer, the withdrawal threshold to mechanical stimulation increased to above the pre-CCI level. The anti-alodynic effect gradually decreased, but significant increase of the threshold continued to four weeks after the gene transfer.

**Discussion:** The patients with neuropathic pain often unresponsive to conventional treatments such as NSAIDs. Stimulations of opioid-receptors have been established for the treatment of abnormal pain sensations after injuries of central or peripheral nerves. Oral or inavenous application of opioid-receptor agonists are mostly often used for the neuropathic pain which resists conventional treatments. Continuous epidural infusion by intrathecal pumps has been established as more effective application method to the CNS. However, there is also a limitation by its short half life of these agents. Several previous studies encouraged to produce selective and continuous delivery of opioids to the CNS by gene transfer [2]. However, virus infection to the CNS may be dangerous for clinical use because of the risk of meningitis [1]. To avoid a risk of viral vector, we used electroporation, a rapid and safety non-viral gene transfer system. The advantage of non-viral system may be significant for clinical use. Using this method, we successfully produced anti-hyperalgesic and anti-allodynic effects which continued at least 4 weeks after a single intrathecal injection. The transplantation of gene modified autologous macrophages may be a safe, simple and effective method for proenkephalin-gene transfer method for the treatment of neuropathic pain after peripheral nerve injuries.


Data are shown as percentages of pre-CCI level in each animals. For statistical analysis of the data, an analysis of variance (ANOVA) followed by Fisher’s PLSD was used.