In Vivo Gene Transfer into the Periventricular Region by Electroporation

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Abstract

Gene transfer by electroporation was attempted in the normal rat brain. The reporter gene pEGFP-C1 (25 μ g/5 μ l) was injected into the striatum of young adult rats and various square electrical impulses were applied using a pair of electrodes implanted in the striatum. The brains were removed and sliced after 5 days. Histological examination revealed that the high energy impulses caused extensive tissue damage whereas lower energy impulses (200–400 mJ) resulted in the transfection of more than 300 cells per brain, which were widely distributed in the subependymal region of the lateral ventricle and extended long processes into the striatum.

Key words: in vivo gene transfer, central nervous system, electroporation, green fluorescence protein, periventricular region, rats

Introduction

In vivo gene transfer using nonviral vectors is of great interest because of the advantages of low toxicity and nonimmunogenicity. Cationic liposomes can successfully mediate gene transfer in the brain in vivo, but the transfection efficiency is less than that of viral vectors.^{2,4)} Electroporation is another nonviral method for gene transfer, which is well established in cultured cells and generally believed to be more efficient than cationic liposomes. In vivo electroporation for gene transfer was first applied in mouse skin,¹²⁾ followed by cells from other organs, e.g., liver,³⁾ skeletal muscle,¹⁾ testes,¹³⁾ corneal,⁹⁾ embryo,⁵⁾ and melanoma cells.¹¹⁾ Intra-arterial injection of vector followed by electroporation can also successfully transfect grafted glioma cells in the brain, as dominant expression of the reporter gene was found in the endothelial cells in the tumor.⁷⁾ However, the heat generated by the electrical impulses during electroporation is likely to cause trauma in vivo and, therefore, in vivo gene transfer by electroporation has not been applied to normal neuronal tissue. The present study evaluated the efficacy of in vivo gene transfer by electroporation in the normal brain in a rat model.

Materials and Methods

Fourteen-day-old Sprague-Dawley rats were used in the present study. All experiments were conducted according to the Guidelines for Animal Experimentation at the Kobe University School of Medicine. The first set of experiments used large electrodes, 0.5 mm diameter and 4 mm working length, coated with platinum (Unique Medical Imada Co., Tokyo) (Fig. 1 left). The animals (n = 12) were anesthetized by inhalation of halothane in air. Plasmid deoxyribonucleic acid (DNA), pEGFP-C1 (25 μ g/5 μ l), was injected unilaterally into the striatum using stereotactic coordinates from the bregma: +0.2 anteroposterior (AP), 2.7 medial lateral (ML), and 6.0 dorsal ventral (DV). A pair of electrodes was inserted into the ipsilateral striatum at the following coordinates: +1.7 and –1.3 AP, 2.7 ML, and 5.0 mm in depth from the top of the electrode (Fig. 2). The distance between the two electrodes was 3 mm. Immediately after placement of electrodes, a single electrical pulse of 150–300 V for 50 msec was delivered using an electrical pulse generator (Electro Square Porator T820M; BTX, San Diego, Calif., U.S.A.) and a graphic pulse analyzer (Optimizador 520; BTX). The electrodes were left in place for 5 minutes and withdrawn slowly. Five days after in vivo electroporation, the animals were perfused transcardi-

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Fig. 1 Two types of electrodes used in the present study. The large electrode with blunt top shape has 4 mm working length (left). The small electrode with sharp cone shape has 1 mm working length (right).

Fig. 2 Trajectory for the placement of electrodes and injection of plasmid. cc: corpus callosum, DNA: deoxyribonucleic acid, lv: lateral ventricle, str: striatum.

ally with a 4% paraformaldehyde solution. The brain was removed and cut serially into 50 μ m sections using a vibratome. Every other section was observed under a fluorescent microscope and the green fluorescence protein (GFP)-positive cells were

Fig. 3 Photomicrographs of sections after electroporation with large electrodes (A) and small electrodes (C), demonstrating a large hole associated with wide tissue damage made by a large electrode and limited focal damage by a small electrode in the striatum (arrows) (Nissl stain, $\times 20$). Fluorescence photomicrograph (B), demonstrating a large lesion in the striatum due to the trauma of impulse with the large electrode, and green fluorescence protein (GFP)-positive cells adjacent to the margin of the lesion (arrows) ($\times 20$). Fluorescence photomicrograph (D), demonstrating GFP-positive cells in the tract of the small electrode (arrow) ($\times 200$). Photomicrographs, demonstrating the GFP-positive cells extending fine processes (arrows) into the corpus callosum and the striatum, predominantly in the dorsolateral wall of the lateral ventricle (E) and the periventricular region around the lateral ventricle (F) ($\times 200$). Asterisk in (C), (E), and (F) indicates the lateral ventricle.

counted by an observer blinded to the section origin. Selected sections were stained with Nissle or counterstained with Propidium Ionide (Sigma, St. Louis, Mo., U.S.A.) to examine tissue damage.

The second set of the experiments ($n = 40$) used fine and cone-shaped electrodes of 0.25 mm diameter and 1 mm working length, instead of the large electrodes (Fig. 1 right). A pair of modified electrodes was placed in the unilateral striatum using the same coordinates as the first experiments. The electrical pulse voltage varied between 50 and 300 V and the duration of each pulse varied between 5 and 50 msec to investigate the optimum pulse conditions.

Results

In the first experiment, all brains contained less than 40–50 GFP-positive cells per brain. In addition, extensive tissue damage was observed (Fig. 3A, B). In the second experiment, a comparable number of GFP-positive cells were found but the tissue damage was less (Fig. 3C). Interestingly, in addition to the transfected cells in and around the electrode tract (Fig. 3D), GFP-positive cells were widely distributed within the subependymal region of the lateral ventricle with long processes extending into the striatum (Fig. 3E, F). The processes from these cells ran nearly tangentially and the maximum length was approximately 200 μm . Such transfected cells were often found in the dorsolateral corner of the lateral ventricle. The corpus callosum in each brain slice included a few transfected cells with the morpho-

gy of oligodendrocytes (Fig. 4 left).

The optimum electrical energy for transfection was estimated in the range between 200 and 400 mJ [$\text{mJ} = (\text{voltage})^2 \times \text{pulse length (msec)} / 4.2 \times \text{resistance}$]. The number of transfected cells exceeded 300 in each brain using this energy range, although some cells were probably degenerating based on the lack of cytoplasm. The transfected cells were located only around the needle tract with a lower energy range and the number of GFP-positive cells decreased associated with tissue damage with a higher energy. Unexpectedly, application of higher energy resulted in GFP-positive cells in the meningeal cells around the point of needle penetration (Fig. 4 right).

Six animals treated with this optimal range of energy did not demonstrate GFP-positive cells in the brain after 2 weeks, indicating that stable transfection could not be achieved, or that the transfected cells died within 2 weeks. A control study ($n = 5$) injected plasmid DNA at the same coordinate followed by insertion of fine electrodes but no electric impulse was applied, which resulted in no detection of GFP-positive cells in the brain. To assess other sites for plasmid injection, the same dose of DNA was also injected into the lateral ventricle ($n = 12$), and a pair of fine electrodes was placed either in the unilateral striatum as above, or in the bilateral striatum at the following coordinates: +1.7 AP, 2.7 ML, and 5.0 DV. However, histological analysis detected no transfected cells.

Fig. 4 Fluorescence photomicrographs, demonstrating a green fluorescence protein (GFP)-positive cell in the corpus callosum with the morphology of oligodendrocytes (left: $\times 200$), and GFP-positive cells in the meningeal membrane around the point of electrode penetration (arrow) (right: $\times 40$).

Discussion

Direct in vivo transfection of DNA in the brain using cationic liposomes has achieved the expression of the *Escherichia coli* β -galactosidase reporter gene for 9 days in neonatal mice, and although the precise location of the injection was unclear, the transfected cells were frequently detectable near the ventricles.⁸⁾ Similar expression of the X-gal gene was seen for 4 weeks in 7-week-old mice, located mostly in the needle tracts.¹⁰⁾ The present study achieved dominant expression of the gene in the cells of subependymal region in addition to cells around the electrode tracts. The plasmid DNA was injected in the center of the striatum, so the concentration of plasmid is thought to be higher in the striatum than in the subependymal region. Dominant expression occurred in the cells of the subependymal region because the cerebrospinal fluid rather than the brain parenchyma was probably the major channel for the electrical current and the transfection was more efficient in the subependymal region than in the striatum. Injection of plasmid into the ventricle failed to transfect any cells, due to the dilution of plasmid DNA in the ventricle. A higher concentration of plasmid DNA in the cerebrospinal fluid of the ventricle may induce more robust expression of the gene in the subependymal region. The cells transfected with GFP exhibited long processes. Immunostaining did not demonstrate co-expression of glial fibrillary acidic protein, microtubule-associated protein-2, and GFP in a single cell (data not shown), so we could not conclude what type of the cells in the brain were transfected.

In vivo electroporation for gene transfer is currently an unfamiliar method, but various experimental studies are ongoing.⁶⁾ In theory, any type of tissue could be a target for transfection and repeat administrations of DNA are possible. One major drawback for application in brain tissue is the damage due to heat. Our study found that lower electrical power than previously reported is sufficient for brain transfection, in particular, in the cerebrospinal fluid space. In previous studies, endothelial cells of corneal origin or grafted tumors were the preferential target for gene transfer. Electroporation may be a powerful tool for gene transfer in such superficial layers of target organs outside of the central nervous systems.^{7,9)} In the central nervous system, tissue damage caused by the electrical shock is inevitable and the electroporation is not suitable for neuronal regeneration. One potential application is during lesioning surgery for movement disorders. For example, modulation of glutamate kinetics may be possible by transfection of

glutamate transporter in the globus pallidus in patients with Parkinson's disease. Another possible application is treatment of disseminated tumor cells in the ventricle system by transfection with tumor suppression gene. The dura membrane and choroid plexus are other possible targets for electroporation. Genes encoding neuronal trophic factors can be transfected and the proteins efficiently delivered into the cerebrospinal fluid.

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Commentary

In this original article, the authors discussed the effectiveness of electroporation in the preliminary experimental stage as a novel method of gene transfer in the central nervous system. They emphasized the efficacy and safety using small needles for the electric current to transfer with minimum heat damage. This method is characterized as delivery along the cerebrospinal fluid. In recent years, we have controlled the primary lesion in induction therapy by combination of microsurgery, navigation, intraoperative ultrasonography and adjuvant chemoradiation therapy. But there is no obviously effective treatment modality for spreading lesions such as meningeal and subependymal dissemination along the cerebrospinal fluid. This method may be especially effective for superficial disseminated lesions, so I hope this method of gene transfer can be introduced for such pathological conditions in the near future. Of course the authors should present the effectiveness for such lesions in their next paper. I also think this method may be useful as a drug delivery system into the central nervous system. Some new modality using chemotherapeutic agents for disseminated lesions may be based on this method.

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The authors have used in vivo gene transfer using electroporation to express the reporter gene, green fluorescent protein (GFP), in the brains of rats. After appropriate determination of the optimum electrical energy stimulus for transfection, they show that the best stimulus is between 200 and 400 mJ to achieve GFP expression in nearby cells. I assume that the electrical stimulation immediately followed the implantation of DNA, although this is not specifically stated in the text. It would appear that there is a delicate balance between the right energy stimulus to achieve transfection versus too much energy stimulus which results in tissue necrosis. These types of experiments are exceedingly important if in vivo gene transfer is to be used in future "gene therapy" studies. With perfection of the technique, it is hoped that even more cells will be transduced following electrical stimulation, and that the length of time of gene expression can be examined post-transfection. I congratulate the authors on these impressive studies using in vivo gene therapy.

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This is an interesting and important report demonstrating successful gene transfer into the normal brain using the in vivo electroporation technique. Recently, this relatively new method for in vivo gene transfer has become more popular because of its many advantages. Successful treatment of an experimental cancer model with plasmid DNA for HAV-tk gene was achieved and reported using this gene transfer method from another group.¹⁾ This method has the potential to become one of the most efficient techniques for gene transfer into the brain when the machines and electrodes are improved and the optimal conditions are determined. The authors demonstrated that the cells in the subependymal region expressed the transgene more efficiently than those in the striatum even when the plasmid DNA was injected into striatum. Their hypothesis that the cerebrospinal fluid is the major channel for the electrical current is clinically very attractive, but the distribution of injected DNA in the brain at the time of electroporation needs to be investigated to discuss this important result.

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