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High-Efficiency *in Vivo* Gene Transfer Using Intraarterial Plasmid DNA Injection following *in Vivo* Electroporation[1]

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ABSTRACT

A novel method for high-efficiency and region-controlled *in vivo* gene transfer was developed by combining *in vivo* electroporation and intraarterial plasmid DNA injection. A mammalian expression plasmid for the *Escherichia coli lacZ* gene (driven with a SV40 early promoter) was injected into the internal carotid artery of rats whose brain tumors (from prior inoculation) had been electroporated between two electrodes. The *lacZ* gene was efficiently transferred and expressed in the tumor cells 3 days after plasmid injection. However, neither any gene transfers nor any elevated *lacZ* activity was detected in tissues outside the electrodes. The plasmid was not transferred without electroporation. Human monocyte chemoattractant protein-1 cDNA was also transferred by this method, and its long-lasting (3 weeks) expression was confirmed by using the Epstein-Barr virus episomal replicon system. The expressed monocyte chemoattractant protein-1 protein was functional, as evident by the presence of a large number of monocytes in the tumor tissue. This method, "electro-gene therapy," which does not require viral genes or particles, allows genes to be transferred and expressed in desired organs or tissues, and it may lead to the development of a new type of highly effective gene therapy.

INTRODUCTION

Many types of methods and techniques for *in vivo* gene transfer have been developed, and some of them have already been applied in clinical trials. In most cases, genes are transferred into tissues by the infectivity of viral particles. The retroviral system, which is the most widely accepted gene transfer method to date, can achieve highly efficient integration, providing the potential for permanent gene expression. However, the system has some major disadvantages, such as the typically low titer, instability of the viral vector obtained, and the requirement for target cell division for integration and expression (1). Although the adenovirus system can provide more efficient gene transfer and stability of virus, the difficulties in control of target cells and of re-administrations necessitated by the strong antigenicity of the virus are serious problems (2). Furthermore, there are some common drawbacks in viral gene transfer systems. Particularly, the quality control of viral particles for *in vivo* administration is laborious and expensive work (3).

If an efficient and specific transfer method could be developed, naked plasmid DNA is an ideal system for gene transfer. A plasmid-mediated method would be economical and easy because use of this system obviates the necessity to construct viral vectors, establish clones of producer cells, and assess viral titers and the presence of replication-competent helper virus, which has been known to activate passive oncogenes. Also, the transfer procedure could be easily repeated because naked plasmid DNA has little antigenicity to the host body (4, 5).

Electroporation or electrical permeabilization of cell membranes has been developed for the purpose of achieving highly efficient *in vitro* gene and/or drug transfer (6–9). This system provides markedly higher efficiency transfer compared with other nonviral transfer systems, including cationic liposomes. Application of the electroporation to cultured cells is well established, but the use of *in vivo* electroporation has received little attention (10, 11). Recently, the electrical permeabilization method has been applied to *in vivo* drug transfers for cancer treatment, and a clinical trial has been started (12, 13). This method is called electrochemotherapy or electric impulse chemotherapy.

In our study, a mammalian expression plasmid carrying the bacterial *lacZ* gene was intraarterially injected into rats immediately after *in vivo* electroporation (between a pair of electrodes) of rat brain tumor cells that had been inoculated into their brains 10 days earlier. The injected plasmid was efficiently incorporated into tumor tissues and found to provide the expression of the lacZ protein in the cells. Sections from a wide variety of tissues from treated animals were analyzed for evidence of toxic effects. We found no treatment-related toxic reactions nor increased *lacZ* activity in any organs other than those between the electrodes in treated animals. We have also tested for long-lasting expression of the human cDNA encoding the *MCP*-1,[3] a cytokine that gathers macrophages (14–16), in the EBV episomal replicon vector. Immunohistochemical analysis revealed that human MCP-1 was expressed in inoculated tumor cells for at least 3 weeks.

This method will allow us to easily deliver the gene of interest to desired tissues or organs. Many applications, including gene therapy of malignant solid tumors and of metabolic disorders, will become possible. We feel this method could be named "electro-gene therapy."

MATERIALS AND METHODS

Rat Brain Tumor Model. C6 rat glioma cells (1×10^7; American Type Culture Collection, Rockville, MD) were stereotactically inoculated into the right striatum of Wistar rats weighing 150–200 g. In all procedures, animal care was in accordance with institutional guidelines.

Carotid Artery Cannulation, Electroporation, and Plasmid Injection. On the tenth day after inoculation, the tumor-bearing rats were randomized into four experimental groups designated: E+P+(ic), $n = 8$; E+P+(iv), $n = 3$; E+P−, $n = 8$; and E−P+, $n = 7$; where E = electrical pulse, P = plasmid, and ± indicates presence or absence, respectively. The animals of the E+P+(ic) group received a run of electrical pulses, followed by intracarotid plasmid DNA injection. The E+P+(iv) group received i.v. injection of the same amount of plasmid DNA following electroporation instead of intraarterial injection. The E+P− group received electrical pulses without plasmid injection, and the E−P+ group received intraarterial plasmid injection only.

Rats were anesthetized by inhalation of halothane. For intraarterial injection, a microcatheter connected to a 2.5-ml syringe was cannulated into the right internal carotid artery through the external carotid artery. The pterygopalatine artery, a major branch of the internal carotid artery, was coagulated to concentrate injected plasmid into the brain circulation. The animals in the E+P+(iv) and E+P− groups received the same operation without plasmid injection.

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[3] The abbreviations used are: MCP-1, monocyte chemoattractant protein-1; H & E, hematoxylin and eosin; X-Gal, 5-bromo-4-chloro-3-indolyl-β-D-galactoside; EBV, Epstein-Barr virus.

After cannulation, the heads of the rats were fixed onto a stereotaxic operating table. The head skin was cut and retracted. One each burr hole was opened in front and behind of the burr hole used for cell inoculation. A pair of stainless steel electrodes, 0.5 cm long and 0.5 mm in diameter, coated with gold (Unique Medical Imada Co., Tokyo, Japan), was inserted into the brain through the burr holes, and the resistance between the electrodes was measured with a BTX 500 optimizer (BTX, Inc., San Diego, CA). The average distance of the electrodes was 0.5 cm. A series of eight electrical pulses with pulse length ranging from 95–99 μs (17–19) was delivered with a standard square wave electroporator BTX T820 (BTX, Inc., San Diego, CA) in groups E+P+(ic), E+P+(iv), and E+P− as shown in Fig. 1. The voltage was adjusted to deliver 0.5 mA of current between the electrodes based on the results of the premeasured resistivity. The average voltage was 300 V/0.5 cm.

Immediately after they received electrical pulsing [group E+P+(ic)] or a sham operation on the head (group E−P+), 10 μg plasmid, pCH110, a mammalian expression vector of the *lacZ* gene with the SV40 early promoter (20) in 0.5 ml PBS was injected by carotid catheter into the rats with a microinfusion pump for 5 min. In group E+P+(iv), the same amount of plasmid DNA was injected into the tail vein. Previous data indicated that uptake by electroporated cells through membrane pores is not affected by whether the molecules were present during or added after the electroporating pulse (21, 22). Therefore, we preferred to inject DNA immediately after electroporation to supply a higher concentration of DNA into the electroporated region using a smaller amount of DNA. After injection, the catheter was evacuated, and the external carotid artery was coagulated and ligated with silk.

X-Gal Staining. Three days after application of the electrical pulses with/without plasmid injection (intraarterial or i.v.), the bodies of three rats in each group were perfused via the heart with 200 ml of PBS, followed by 200 ml of 4% paraformaldehyde in PBS. Brain, heart, lung, liver, spleen, kidney, and testis were removed and fixed in the fixation buffer overnight. Brains were cut by vibratome into 60-μm thick slices. Other tissues were soaked in sucrose buffer (10% sucrose in PBS) overnight, and the concentration of sucrose was gradually elevated to 30%. Tissues were then frozen embedded in OCT Compound (Miles, Inc., Elkhart, IN), cut into 10-μm thick slices with a cryotome, and stained with H & E to observe histological change. Sections of the rat brain, including electrode tracts, were also stained with H & E for histological observation.

Expression of β-galactosidase was detected using X-Gal histochemical stain (23). Staining tissues that express β-galactosidase with X-Gal turns cells blue. When an indoyl is liberated from X-Gal by the catalytic activity of β-galactosidase, its subsequent oxidization and self-coupling forms an indigo blue derivative. Therefore, only cells that accepted the injected plasmids and cells with endogenous β-galactosidase can be stained by this method and thus be discriminated from other cells, using light microscopy.

Construction of the Expression Vector of the Human MCP-1 cDNA. To test long-lasting transgene expression by our method, we selected another marker gene, human *MCP-1* cDNA (15, 16). This cytokine is produced at a low level in normal tissues and at a high level in some kinds of tumors, including brain tumors, and has a significant role in intratumoral monocyte infiltration (24, 25). Since the antitumor effect of this cytokine is reported based on the results of the transfection experiment (26), we selected this gene as a candidate transgene for the gene therapy of brain tumors. The mammalian expression vector pCEP4 (Invitrogen, San Diego, CA) was used in this study. This vector system includes discrete DNA elements, the EBV origin of replication, and EBV nuclear antigen-1. Because these elements together confer an episomal replicon capacity to DNA circles in an array of human cell types, this vector not only offers an expeditious means for achieving amplification of transfected genes but also bypasses *cis* effects upon promoter function at chromosomal integration sites. The 700-bp fragment of human MCP-1 cDNA was ligated into the unique *Bam*HI/*Hin*dIII sites of the pCEP4 vector (pCEP4/hMCP-1) with T4 DNA ligase (New England Biolabs, Beverly, MA). Thirty μg of the resulting plasmid pCEP/hMCP-1 were transferred into five tumor-bearing rats in the same manner with pCH110.

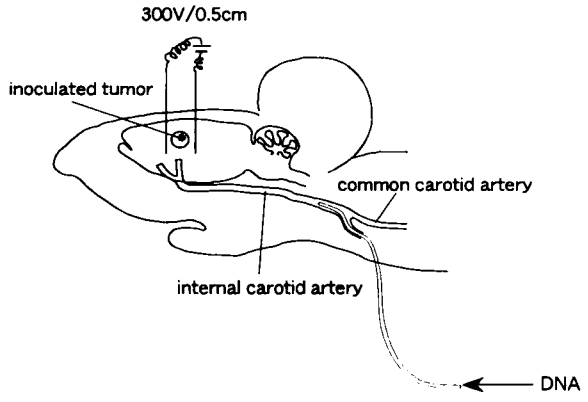

Fig. 1. Schematic expression of the method for electro-gene therapy.

Immunohistochemistry of Human MCP-1 Protein in Rat Brain. Three weeks after the electro-gene transfer procedure, tumors in rat brain were stained by the indirect immunoperoxidase method. Cryostat sections were cut at a thickness of 6 μm and air dried. To inhibit endogenous peroxidase activity, the sections were first immersed in 5 mM periodate solution for 10 min, washed with PBS, and then placed in a 3 mM sodium borohydrate solution for 30 min (27). They were then incubated with optimally diluted monoclonal IgG anti-human MCP-1 (28). After being washed with PBS, they were covered with peroxidase-conjugated, species-specific goat antimouse immunoglobulin (Fab'2) diluted 1:100 (Amersham, Arlington Heights, IL) for 60 min. Peroxidase activity was visualized by incubation of the sections with 3,3'-diamino benzidine [0.5 mg/ml Tris-HCl buffer (pH 7.6) containing 0.01% H_2O_2; Sigma Chemical Co., St. Louis, MO] for 10 min.

Measurement of β-Galactosidase Activity. For this experiment, each rat's body was perfused with 200 ml of ice-cold PBS. From the brain, the region including the inoculated tumor and the contralateral brain were cut out and immediately frozen. The organs (heart, lung, liver, spleen, kidney, and testis) were sampled, and about 100 mg of each tissue were minced, immediately frozen in a 1.5-ml Eppendorf tube in dry ice powder, and stored at −80°C until used. Before sample homogenization, 150 μl of ice-cold 0.25 M Tris-HCl (pH 7.8) were added to each tube. After repeating the freezing-thawing procedure three times, the samples were centrifuged for 30 min at 4°C. The protein concentrations of the resulting 150-μl supernatants were determined using a BCA kit (Pierce, Rockford, IL) and used for β-galactosidase assay. The assay was carried out on microtiter plates. Ten μl of supernatant were added to 290 μl of Z buffer [60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgCl_2$, and 50 mM β-mercaptoethanol] in a microtiter plate well and incubated at 37°C for 10 min. Then, 30 μl of prewarmed 15 mM chlorophenol red-β-D-galactopyranoside in Z buffer were added to each well and incubated at 37°C for 60 min (29). The reaction was stopped by adding 75 μl of 1 M Na_2CO_3 to each well, and the color reaction was quantitated in an ELISA reader set at 570 nm. The β-galactosidase activity was expressed as: (absorbance at 570 nm)/mg protein × 10,000. Comparisons of values were analyzed by Student's *t* test with $P < 0.05$ considered to indicate statistical significance.

RESULTS

Expression of the Intraarterially Injected *lacZ* Gene in Rat Brains. We performed *in vivo* electroporation of inoculated brain tumors in rats, followed by intraarterial injection of plasmid DNA containing the *lacZ* gene. To evaluate the efficiency of gene transfer, we performed X-Gal staining on slices of the treated rat brains. Fig. 2, *A-D*, shows the representative results of X-Gal staining of rat brain on the third day following electroporation and intraarterial plasmid injection [E+P+(ic) rats]. A restricted region centered along the border between inoculated tumor cells and adjacent brain stained blue, indicating expression of *lacZ* (Fig. 2*A*). The high magnification picture shows dense staining of the border region and vascular wall and light staining of the region inside the tumor tissue (Fig. 2, *B* and *C*). The high magnification of X-Gal staining with H & E counterstaining clearly demonstrates that the tumor cells but not the adjacent tissue highly expressed the lacZ protein (Fig. 2*D*). The density of stained cells is higher in the region close to tumor vessels. No staining was observed outside of the tumor border (Fig. 2*D*, *lower right corner*) in this slice.

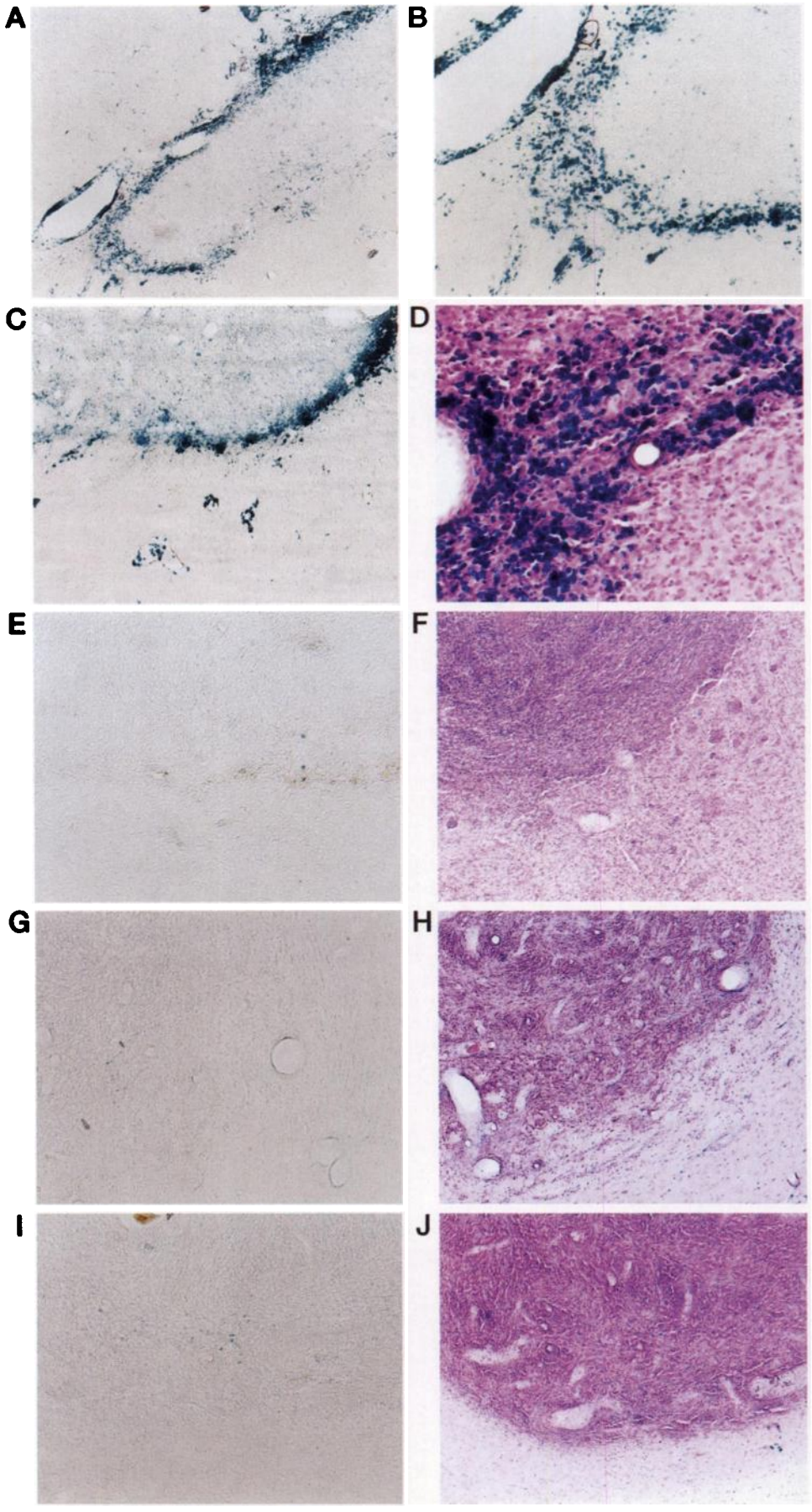

Fig. 2. Microscopic visualization of rat brains treated with electro-gene transfer. X-gal staining of brains with tumors. Photomicrograph demonstrates β-galactosidase-transduced cells in rat brains 3 days after the electro-gene transfer [E+P+(ic) group: *A, B, C,* and *D*] and control procedures [E+P− group: *E* and *F*; E−P+ group: *G* and *H*; E+P+(iv): *I* and *J*]. *A*, sagittal section of E+P+(ic) rat brain; X-Gal staining, ×20. *B*, higher magnification of *A*, ×100. *C*, coronal section of the another E+P+(ic) rat brain; X-Gal staining, ×40. *D*, high magnification of X-Gal staining with H & E counterstain of the another E+P+(ic) rat brain, ×200. *E*, coronal section of E+P− rat brain; X-Gal staining, ×40. *F*, X-Gal staining with H & E counterstain of E+P− rat, ×40. *G*, coronal section of E−P+ rat brain; X-Gal staining, ×40. *H*. X-Gal staining with H & E counterstain of E−P+ rat brain, ×40. *I*, coronal section of E+P+(iv) rat brain; X-Gal staining, ×40. *J*, X-Gal staining with H & E counterstain of E+P+(iv) rat brain, ×40.

E+P− (electroporation only) rat brain shows no X-Gal-positive cells, even in the border region of inoculated tumor tissues (Fig. 2, *E* and *F*). Only several cells inside vascular structures expressed in E−P+ (plasmid injection only) rat brain (Fig. 2, *G* and *H*). Furthermore, electroporation followed by i.v. plasmid injection could deliver the *lacZ* gene into a few tumor cells, including vascular wall [E+P+(iv) rats; Fig. 2, *I* and *J*].

Expression of the Human *MCP-1* Gene 3 Weeks after Electroporation and Microscopic Observation of the Electrode Tract. The human *MCP-1* cDNA expression plasmid with the EBV episomal replicon system was transferred into the inoculated rat brain tumor by electro-gene transfer. Long-lasting transgene expression was examined by immunohistochemical study on frozen sections of the rat brains at 3 weeks after the transfer procedure, at which time the tumor cells were shown to be expressing human MCP-1 protein (Fig. 3, *A* and *B*). Because the antihuman MCP-1 monoclonal antibody used in this study does not cross-react with endogenous rat MCP-1 protein (28), immunoreactive cells express human MCP-1 encoded by the transferred plasmid. Significantly, large numbers of macrophages and lymphocytes were observed in tumor tissues, especially in the peripheral area (Fig. 3*B*, *lower left corner*). The antitumor effects of expressed MCP-1 and infiltrated host cells are now under investigation. No expression of human MCP-1 protein was detected in E+P− (electroporation only) rats (Fig. 3*C*).

The tracts made by electrode insertions were filled with astrocytes and lymphocytes, but there were no reactive changes in the surroundings (Fig. 3*D*), consistent with a previous report (17). No adverse clinical effects, such as seizure and paresis, were observed in rats in any groups during the 3-week follow-up period.

β-Galactosidase Activities in Rat Brain and Other Organs Outside Electrodes. To quantify the efficiencies of gene transfer and expression achieved by the electro-gene transfer, we measured the *lacZ* enzyme activities in homogenates of the various tissues. The region that included inoculated C6 cells expressed statistically significant higher activities in E+P+ rats than in E+P− and E−P+ rats, whereas the contralateral brain does not have high activities (Fig. 4*A*). A previous study demonstrated that systemic i.v. injection of plasmids mixed with cationic liposomes allowed systemic expression of the transferred gene (30). This nonspecific delivery of injected plasmid is a potential major problem in our system using intraarterial injection. Therefore, we examined whether the organs outside electrodes showed expression of the transferred gene. We measured the *lacZ* enzyme activities in homogenates of the major organs such as heart, lung, liver, spleen, kidney, and testis and compared the activities in DNA-injected rats [E+P+ (ic), $n = 5$; E−P+, $n = 4$] with those in DNA noninjected rats (E+P−, $n = 5$). Each organ had endogenous enzyme activity at various levels. Although kidneys in electroporation and DNA-injected rats expressed relatively high β-galactosidase activities, the difference between these levels and those seen in the groups not injected with DNA was trivial and not significant ($P = 0.281$). The enzyme activities in other organs were at the same levels in both groups (Fig. 4*B*).

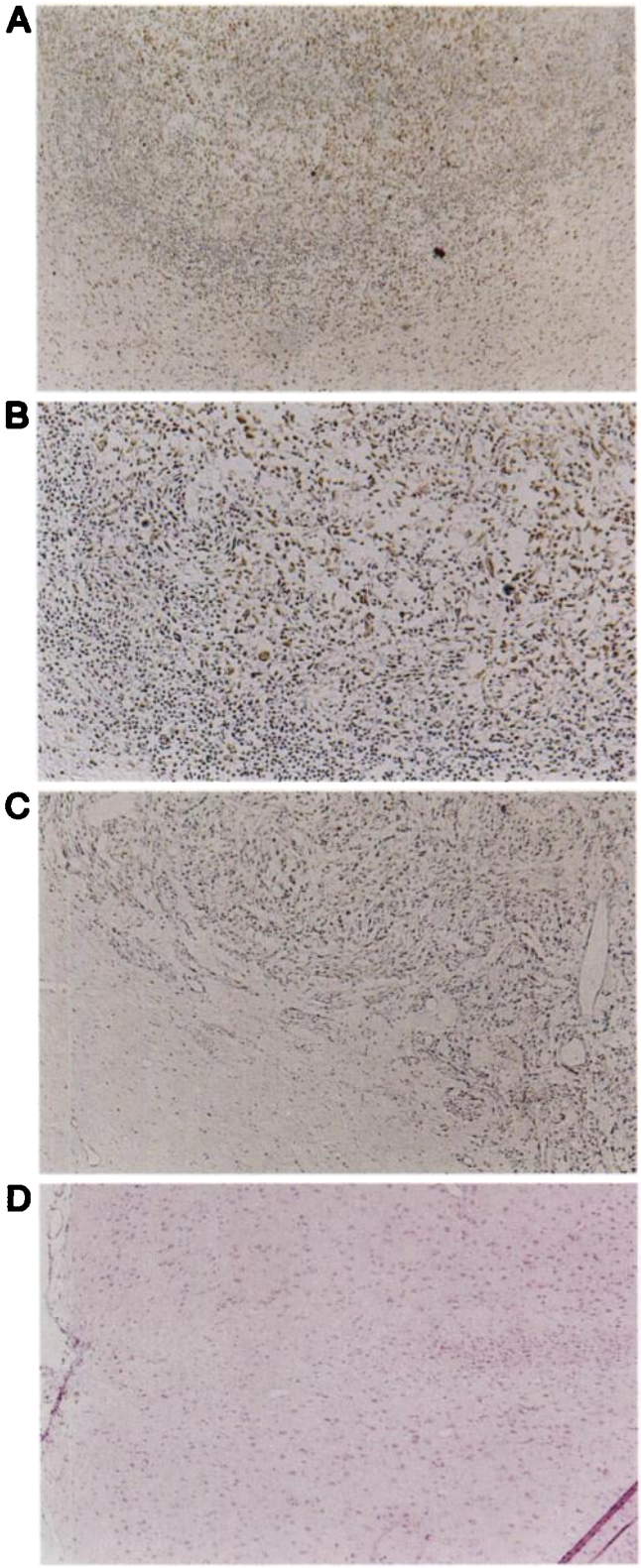

Fig. 3. Immunohistochemical detection of the human *MCP-1* gene product in tumor cells inoculated in rat brain. The human MCP-1 expression vector, pCEP/hMCP-1, was intraarterially injected after electroporation into rats with brain tumors. Rats were sacrificed 3 weeks after the transfer procedure. The brain slice, including tumor tissue, was subjected to immunohistochemical staining with antihuman MCP-1. *A* and *B*, immunohistochemical staining of human MCP-1 protein of E+P+(ic) rat brain. *A*, most of the residual tumor cells expressed human MCP-1 protein. A large number of monocytes are observed in tumor tissue, especially in the peripheral area of the tumor; ×30. *B*, higher magnification of *A*; ×100. *C*, immunohistochemical staining of E+P− rat brain (electroporation only). Human MCP-1 protein was not detected; ×40. *D*, H & E staining of the rat brain where the electrode was inserted 3 weeks before. The tract is filled with astrocytes and lymphocytes; ×100.

DISCUSSION

This study demonstrates that the novel gene transfer method using *in vivo* electroporation followed by intraarterial plasmid DNA injection can provide efficient and region-controlled gene transfer. Because intraarterial plasmid injection alone could not transfer the gene into the tumor, *in vivo* electroporation was essential for the efficient transfer of the naked plasmid DNA. Furthermore, electroporation followed by i.v. injection could not efficiently transfer the plasmid.

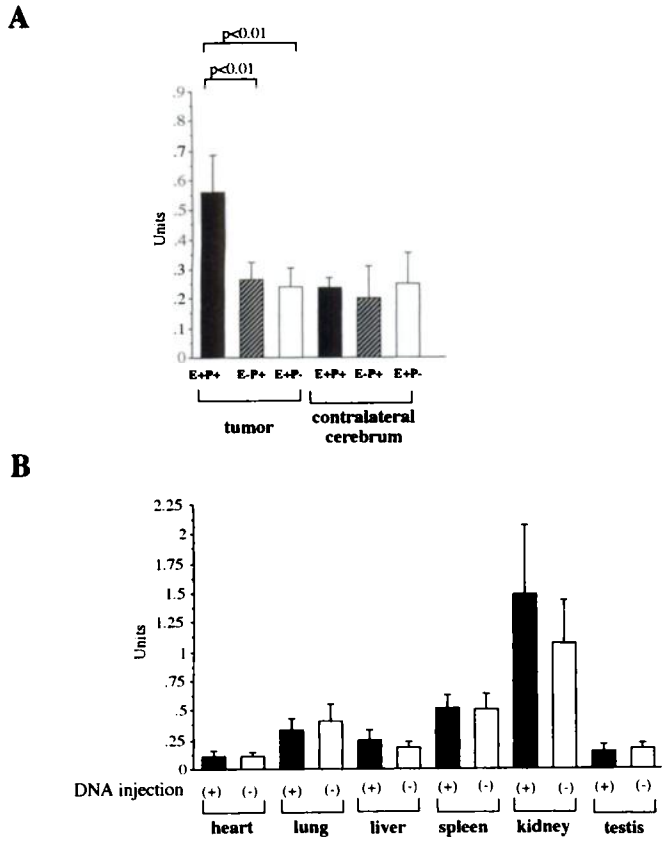

Fig. 4. β-Galactosidase activities in rat brain and other organs. Rats were sacrificed 3 days after the *lacZ* gene transfer. Brain, including inoculated tumor and contralateral brain, were cut out and immediately frozen. Major organs (heart, lung, liver, spleen, kidney, and testis) were excised, and about 100 mg of each tissue were minced and immediately frozen. β-Galactosidase activity was measured by using lysates of homogenized tissues. The β-galactosidase activity was expressed as: (absorbance at 570 nm)/mg protein × 10,000. *A:* β-galactosidase activities in brains. ■, E+P+ rats (electroporation and intraarterial plasmid injection), $n = 5$; ▨, E−P+ rats (intraarterial plasmid injection only), $n = 5$; □, E+P− rats (electroporation only), $n = 4$. *Bars*, SD. *B:* β-galactosidase activities in other organs. ■, plasmid injection + [E+P+(ic) and E−P+ rats], $n = 9$; □, plasmid injection − (E+P− rats), $n = 5$. *Bars*, SD.

This suggests that regionally high DNA concentration in electrically pulsed tissue is a crucial factor for efficient gene transfer.

Injected DNA was distributed in the whole tumor tissue, while the transfer efficiency was uneven within tumor tissue. This efficient transfer could be achieved only by intravascular, especially intraarterial, delivery of the gene. The fact that the transgene was highly expressed in the border region between tumor and adjacent brain, where tumor neovascularization is abundant, may support this hypothesis. The *lacZ* gene was not well transferred into normal brain cells except for cells in vascular walls, even inside the field between the electrodes. It may be partly explained by the differences in vascular structure between normal brain and tumor tissue. Capillaries in tumor tissue may demonstrate cellular fenestrations, wide intercellular junctions, pinocytotic vesicles, and infolding of luminal surfaces (31). By these significant anatomical changes, molecules normally excluded by the blood-brain barrier can penetrate the structure (31). Furthermore, the expression efficiency of transgene in rapidly proliferating tumor cells may be better than in normal brain cells by its demethylation of plasmid DNA. When tumor size is big enough, by inserting electrodes inside tumor tissue, the gene transfer into vascular structure in the normal brain could be avoided.

Zheng and Chang (32) demonstrated that high-efficiency gene transfection can be obtained by directly electroporating cultured mammalian cells in their attached state using a pulsed radiofrequency electric field. Over 80% of the adherent COS-M6 cells took up plasmid DNA and expressed the *lacZ* gene, whereas the transfection efficiency was less than 20% when the M6 cells were electroporated in suspension (32). They speculated that the high-efficiency transfer into attached cells is explained by the characteristics of the attached cells, which have the higher surface:volume ratio compared to trypsin-EDTA treated suspension cells, and the intact submembrane cytoskeleton system.

Because our *lacZ* gene expression system used in the present study was designed to test for transient expression, we measured the β-galactosidase activity at the third day after the electrotransfer procedure. Long-lasting transgene expression was investigated by using the EBV episomal replicon vector, which has human *MCP-1* cDNA. The transferred *MCP-1* cDNA had been expressed in tumor cells for 3 weeks. Furthermore, the expressed MCP-1 protein seemed functional since a large numbers of monocytes were observed in the tumor tissue.

There had previously been some trials of gene transfer using plasmid DNA, such as local injection of mammalian expression plasmids, to produce local expression of a target gene and genetic immunization (33). Also, muscle tissue has been reported to be able to take up injected plasmid DNA efficiently (34), and this uptake was enhanced if the muscle regeneration was induced by the myotoxic local anesthetic bupivacaine (35). Zhu *et al.* (30) reported that systemic i.v. injection of plasmids mixed with cationic liposomes was nontoxic, which is consistent with other reports (36, 37). However, the distribution of the injected DNA by this system was very wide and nonspecific, and the amount of plasmid DNA injected for tissue expression in their study was extremely high. To obtain high levels of chloramphenicol acetyltransferase gene expression, more than 100 μg DNA/20 g mouse tissue (5 μg/g body weight) were injected. In our method, the amount of DNA for injection could be reduced to 10 μg plasmid/200 g rat tissue (0.05 μg/g body weight) by combining *in vivo* electroporation and intraarterial plasmid DNA injection. Although we observed very specific and efficient transfer of injected plasmid using X-Gal staining, potential major limitations of this method could have been nonspecific delivery of injected plasmid outside the field between the two electrodes, as Zhu *et al.* (30) observed for their DNA:liposome complex injection, injury to brain tissues by insertion of the electrodes, and application of high voltage lasting only a fraction of a millisecond. However, our data on the measurement of the enzyme activities in major rat organs with plasmid injection proved that the gene was not transferred outside of the vicinity of the electrodes. Consistent with our data, Nabel *et al.* (36) observed no abnormal pathology in histological examinations after systemic (i.v. and intraarterial) injection of plasmids encoding several different genes, even with plasmid-liposome complexes, into rabbit and pig. They also showed that plasmid was not detected in testis or ovary (36). Recently developed materials and techniques for endovascular procedures allow us to catheterize tumor-feeding arteries super-selectively, leading to a high concentration of injected plasmid DNA to the electrically pulsed region. This technique drastically cuts down the total amount of plasmid necessary for injection, greatly reducing the probability of nonspecific gene transfer.

Bleomycin administration by electrochemotherapy using *in vivo* electroporation was successful for treatment of both inoculated and spontaneous tumors in animals (13, 17). Salford *et al.* (17) applied this method for treatment of brain tumor in an animal model system. No adverse effects were detected during the month following electroporation, which was performed with 8 to 12 exponential 400-V pulses with a time constant at 325 μs. Likewise, we did not observe any clinical side effects, such as seizure or abnormal behavior, during the 3-week follow-up period under our experimental conditions. Histo-

logical examination also revealed no significant pathological changes, even in the regions of electrode insertion.

Further study is essential to determine optimal conditions for gene transfer, such as strength of electric current in electroporation, dosage of plasmid DNA for injection, timing of DNA injection, and proper vector systems. Our preliminary data indicate that *lacZ* activities expressed inside tumor tissue correlates with the strength of the current used for electroporations (data not shown). To avoid tissue injury by electric impulse, optimum electrical parameters for adequate gene transfer should be determined. Under our conditions of experimentation, the current was kept very low and pulse length was relatively short (less than 0.1 ms); yet, we achieved a significant amount of gene transfer and transgene expression. Our novel *in vivo* gene transfer method is a simple procedure and can solve some of the critical drawbacks of the present gene transfer techniques, thus providing a new strategy for gene therapy.

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