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Distribution of Adenoviral Vector in Brain after Intravenous Administration

The delivery of transgenes to the central nervous system (CNS) can be a valuable tool to treat CNS diseases. Various systems for the delivery to the CNS have been developed; vascular delivery of viral vectors being most recent. Here, we investigated gene transfer to the CNS by intravenous injection of recombinant adenoviral vectors, containing green fluorescence protein (GFP) as a reporter gene. Expression of GFP was first observed 6 days after the gene transfer, peaked at 14 days, and almost diminished after 28 days. The observed expression of GFP in the CNS was highly localized to hippocampal CA regions of cerebral neocortex, inferior colliculus of midbrain, and granular cell and Purkinje cell layers of cerebellum. It is concluded that intravenous delivery of adenoviral vectors can be used for gene delivery to the CNS, and hence the technique could be beneficial to gene therapy.

Key Words: *Adenoviridae; Central Nervous System; Green Fluorescent Protein; Gene Therapy*

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INTRODUCTION

Introduction of genes encoding proteins of potential therapeutic value into the central nervous system (CNS) may be the most effective method for treating CNS disorders such as brain cancer, Huntington disease, and Alzheimer disease. To achieve this goal, effective transfer and stable expression of the transgenes in the CNS are essential. To date, several different delivery methods have been demonstrated using protein/DNA complex, mechanical administration, liposomes, and recombinant viral vectors (1). Introduction of foreign genes in terminally differentiated cells like CNS neurons has been practically a highly challenging task, but the field of gene therapy of the CNS has been greatly expanded due to the availability of several recombinant viral vectors. Such viral vectors include adeno-associated virus (2-4). *Herpes simplex* virus (5, 6), vaccinia virus (7), and retrovirus (1, 8, 9), and all of these vectors have successfully modified gene function in the CNS. Among these viral vectors, adenoviral vectors have been extensively used for the delivery of genes in the CNS. Several methods of delivering the viral vectors have been utilized, while vascular delivery being the most recently introduced (10, 11). This is different from other methods, and can optimize delivery to the CNS without multiple invasive procedures.

The main aim of the present study was to characterize reporter gene expression in the CNS after intravenous injection of adenoviral vectors containing green fluorescence protein (*GFP*) genes, and thus provides a basis for developing gene therapy of the CNS.

MATERIALS AND METHODS

Viral vector

The virus vector used in this study was a recombinant type 5 adenovirus vectors (Ad5) with disruption of the E1 and E3 regions, and contained the *GFP* gene placed under the control of the cytomegalovirus (CMV) promoter. It was purchased from Q-Biogene (Montreal, Canada). Viral stocks were generated in 293 cells, concentrated by ultracentrifugation, titered by plaque assays (12), and stored in PBS containing 10% glyc-

erol (v/v).

Mice and adenoviral vector transfer

Each of the thirty adult, female BALB/c mice (8-12 weeks old, 18-21 g; Charles-River Laboratory, Tokyo, Japan) were injected with 0.3 mL of the virus stock (a total of 2×10^8 pfu) via the tail vein. For the controls, only PBS containing 10% glycerol (v/v) was injected. Five animals were sacrificed (by cervical dislocation) at each point, 3, 6, 9, 14, 21 and 28 days after intravenous injection.

Immunohistochemical staining

The brains were extracted, fixed with 4% paraformaldehyde in PBS (pH 7.4) and embedded in paraffin. For immunohistochemical analysis, the brains were sectioned at 10 μm in the sagittal plane. Immunohistochemistry was performed following the Vectastain ELITE protocol (Vector Laboratories, Bulingame, U.S.A.) as previously reported (13). Anti-GFP antibody (Molecular Probes, Eugene, Oregon U.S.A.) was used at a dilution of 1:3,000. Primary antibody was detected using an avidin-biotin-peroxidase kit (Elite kit, Vectastain) and was visualized with diaminobenzidine. Immunofluorescence also was performed, as previously described (14), using rabbit anti-GFP, mouse anti-NST (Neuron Specific Tubulin) (BabCo, Richmond, CA, U.S.A.) and anti-GFAP (Santa Cruz Biotech. Inc., Santa Cruz, CA, U.S.A.) antibodies. Anti-NST antibodies recognized β-tubulin type III, that is specifically expressed in neurons, whe-reas anti-GFAP antibodies recognized glial filament associated proteins expressed exclusively in glial cells. Secondary antibodies included FITC-conjugate (for GFP) and rhodamine-conjugate (for NST and GFAP) (Jackson Immuno-Research Laboratory, West Grove, PA, U.S.A.) and were used at dilutions of 1:50 and 1:100, respectively.

RESULTS

GFP was expressed in the brain after intravenous injection of the adenoviral vectors containing the *GFP* gene. Expression was localized to the hippocampus of cerebral neocortex, inferior colliculus of midbrain, and granular cell and Purkinje cell layers of the cerebellum. In particular, CA regions of the hippocampus showed robust expression of GFP (Fig. 1A), whereas no expression was observed in dentate gyrus. GFP expression in the CA regions is highly localized to pyramidal cell layers (Fig. 1A). Dendritic processes bearing GFP expression were also observed. In the midbrain, GFP expression was found in inferior colliculus, where it appeared in scattered cells. In the cerebellum, GFP expression was highly localized to granular cell and Purkinje cell layers (Fig. 1B). Some GFP expression was observed in molecular layers, representing possible projections of granular cells or Purkinje cells into these layers. The expression of GFP in the brain changed with time after injection of viral vectors (Fig. 1B). GFP expression was first observ-

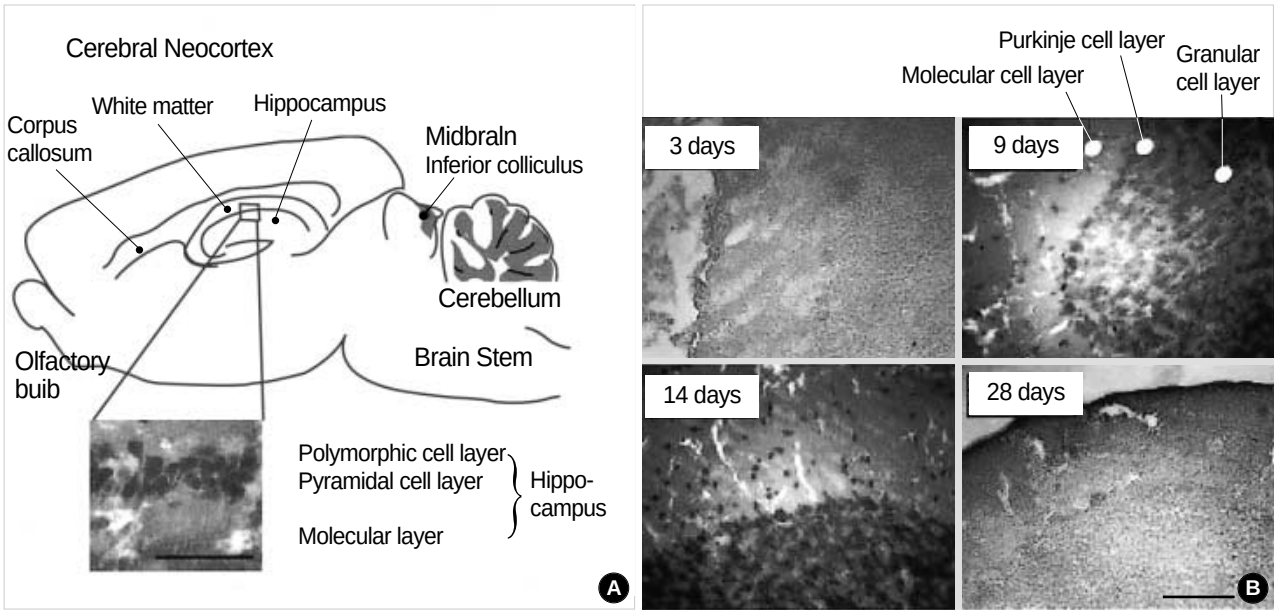

Fig. 1. *GFP* gene expression in the adult mouse brain. (A) Illustration of mid-line sagittal section of a mouse brain. Immunoreactivity of GFP in hippocampal CA1-3 regions of cerebral neocortex is clearly visualized under 40× magnifications. The represented sample was acquired 14 days after injection. (B) Time course of *GFP* gene expression in the cerebellum. GFP is expressed in granular cell layer and Purkinje cell layer of cerebellum. The expression of GFP was transient. The expression was peaked in 14 days after injection of viral vectors. Number of days indicated the day that the subjects were sacrificed after injection. Scale bars represent 100 μm.

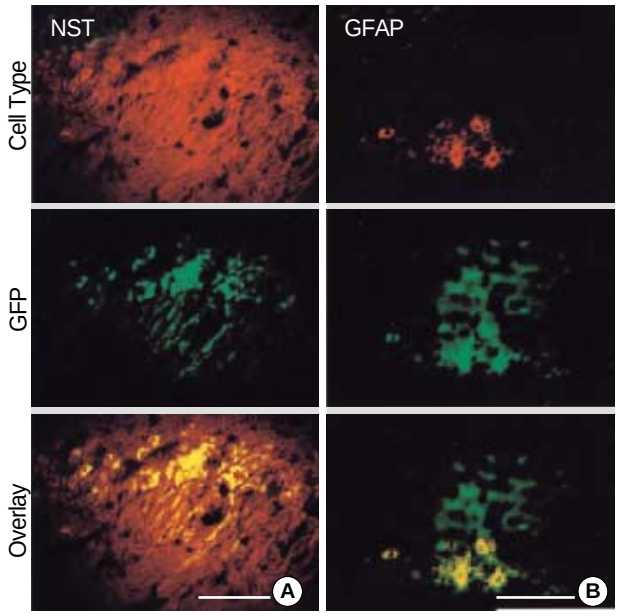

Fig. 2. *GFP* gene expression in neurons and neuroglia. Intravenously introduced adenoviral vectors were transfected in either neurons or glial cells. Neurons in CA1-3 regions of the hippocampus are visualized in red (A). The projections of dendritic processes into molecular layers are also visualized. *GFP* genes are highly expressed in some of these neurons. Merged image demonstrated the expression of GFP in these neurons. Glial cells in neocortex are visualized by GFAP (B). Transfections of adenoviral vectors in some glial cells were visualized in the merged image. The represented sample was acquired 14 days after injection. Scale bars represent 50 μm.

ed from 6 days after injection and maintained intensely up to 14 days. Then, the expression was decreased and was barely detectable after 28 days of injection.

We also investigated if injected viral vectors could infect either neurons and/or neuroglia and express GFP in these cells. Neurons were immunohistochemically detected by anti-NST antibodies (TuJ1), whereas neuroglia were identified by anti-GFAP antibodies. The viral vectors used successfully infected both neurons and neuroglia, and GFP was expressed in both cell types (Fig. 2).

Expression of GFP in the heart, intestine, kidney, liver, lung, and spleen were also examined. GFPs are well and most frequently expressed on the lung among all examined organs. GFP is highly expressed on the splenic trabecular area, renal tubules and intestinal microvilli, but it is very sparsely expressed on the splenic pulp area, renal glomerulus, liver parenchyma and heart. No specific pathologic lesions possibly related with adenovirus administration are observed on microscopic examination of all examined organs including the brain (15).

DISCUSSION

Our observations agree with the recent report from Smith et al. (16) demonstrating a promoter dependent transfection of the adenoviral vectors in vivo and in vitro. The adenoviral vectors containing the CMV promoter were transfected into CA regions and into pyramidal neurons of the rat hippocampus, whereas minimal transfection was showed in the dentate gyrus (16). In contrast, RSV promoter produced higher transfection in the dentate gyrus and granular cells (16). Thus, selection of the promoter could be a critical consideration for the design of adenoviral vectors for cell- or region-specific gene delivery to brain.

It may have been expected that the blood-brain barrier (B BB), formed by the tight junctions of capillary endothelial cells in the brain, would hinder the passage of most polar molecules, including viral vectors (10). Consequently, to increase vector delivery to brain by intracarotid artery infusion with osmotic disruption of the BBB is used for the purpose of facilitating transfection of viral vectors from blood to brain (17). Importantly, however, we demonstrated that the intravenously injected adenoviral vectors were able to enter the brain with an intact BBB, although the adenoviral vectors used are much larger than the open pore size of even disrupted BBB (10). It would seem possible, therefore, that some unknown mechanism(s) could enable the adenoviral vectors to cross the barrier. This is an interesting phenomenon, and further studies may facilitate the development of more efficient delivery systems.

In this study, we demonstrated that intravenously injected adenoviral vectors containing the CMV promoter are able to cross the vascular epithelium in the brain, and infect neurons or neuroglia without disruption of BBB. Thus, intravenous delivery of recombinant adenoviral vectors is feasible for the gene transfer to particular regions and cell types in the CNS. Such an approach could, therefore, be a useful tool for gene therapy of CNS disorders.

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