Recombinant Adeno-associated Virus-mediated Gene Delivery to the Central Nervous System

Toshiyuki Kurai\(^1\)\(^2\) and Takashi Shimada\(^1\)

\(^1\)Department of Molecular and Medical Genetics, Graduate School of Medicine, Nippon Medical School
\(^2\)Department of Vision and Ophthalmology, Graduate School of Medicine, Nippon Medical School

Recombinant adeno-associated virus (rAAV) is capable of highly efficient gene transfer and long-term expression of transgenes in nondividing cells. Accordingly rAAV is a useful vector system for gene therapy of neurological diseases. We are examining the feasibility of AAV-mediated gene therapy of metachromatic leukodystrophy (MLD), which is a lysosomal storage disease caused by the deficiency of arylsulfatase A (ASA) and is characterized by deposition of sulfatide throughout the brain. In the present study, we generated AAV serotype1-based vectors expressing green fluorescent protein (GFP) (AAV1-GFP) or ASA (AAV1-ASA). When AAV1-GFP was stereotactically injected into the CA3 region of the hippocampus in BL6 mice, cells strongly positive for GFP were detected in the area of injection (Fig. 1). In addition, GFP signals were seen throughout the hippocampus even on the side opposite the injection, indicating efficient axonal transport of intracellular GFP molecules to the contralateral hemisphere. Next, AAV1-ASA was injected into the hippocampus of ASA-knockout mice (MLD mice). Detailed immunohistochemical examination revealed that ASA molecules were detected mainly in neuronal cells and localized in the perinuclear area (Fig. 2). Levels of ASA activity and the

---Photogravure---

Fig. 1

Correspondence to Toshiyuki Kurai, Department of Biochemistry and Molecular Biology, Nippon Medical School, 1–15 Sendagi, Bunkyo-ku, Tokyo 113–8602, Japan
extent of ASA distribution could be significantly enhanced by co-expression of formylglycine-generating enzyme, a recently identified activator for sulfatase. These results demonstrate that direct injection of AAV1 vector into the brain is a highly promising approach for gene therapy of MLD.

**Fig. 1** Distribution of GFP in the brain after direct injection of AAV1 vector into the hippocampus. GFP signals were detected in the hippocampus bilaterally.

**Fig. 2** Localization of ASA in neuronal cells after AAV-mediated expression. Confocal microscopic examination shows that ASA is localized in the perinuclear area. (A) ASA immunostaining; (B) GFP signal; (C) Merged picture.