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Targeted Gene Delivery to Selected Liver Segments *Via* Isolated Hepatic Perfusion

Hiroyasu Kinoshita, M.D.,*,† Atsushi Watanabe, M.D., Ph.D.,* Sanae Hisayasu, Ph.D.,* Satoru Suzuki, M.D., Ph.D.,* and Takashi Shimada, M.D., Ph.D.*,[1]

*Department of Biochemistry and Molecular Biology; and †Division of Thoracic Surgery, Nippon Medical School, Tokyo, Japan

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Background. **Development of targeted gene transfer technologies is essential for *in vivo* gene therapy. In this study, we examined the feasibility of physically targeting an adenoviral vector to selected liver segments in rats by isolating the hepatic perfusion (IHSP) and clamping the portal vein between the upper and lower segments.**

Materials and Methods. **The rats were divided into two groups: IHSP group and the inferior vena cava (IVC) group. The adenoviral vector, which harbored the β-galactosidase (β-gal) gene, was administered *via* the portal vein, after which unbound vector particles were washed out with phosphate-buffered saline (PBS) and removed *via* the cannulated inferior vena cava (IVC) in IHSP group, while the IVC group received the transgene directly via the IVC without isolation of the hepatic perfusion.**

Results. **With this configuration (IHSP group), >99% of the β-gal activity was limited to the targeted hepatic lobes, findings which were confirmed by histochemical staining with X-gal. We also found there to be significant differences in transgene expression among the hepatic lobes in the IVC group.**

Conclusions. **Taken together, these results indicate that the IHSP technique is useful for local gene delivery to selected liver segments, and that when evaluating the efficacy of IHSP in the treatment of liver disease (e.g., nonresectable tumors), interlobar differences must be given careful consideration to ensure that sufficient drug or vector is delivered to all targeted hepatic lobes.**

[1] To whom correspondence and reprint requests should be addressed at Department of Biochemistry and Molecular Biology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo, Japan, 113-8602. E-mail: tshimada@nms.ac.jp.

Key Words: **isolating hepatic perfusion; adenovirus vector; gene targeting; hepatic lobe; gene therapy; chemotherapy; rat.**

INTRODUCTION

The liver is often thought of as a potential target for gene therapy because many liver disorders that are either inherited (e.g., hemophilia and hypercholesterolemia) and acquired (e.g., hepatitis and cancer) ultimately will require gene therapy for cure. To date, there have been a number of trials involving both *ex vivo* and *in vivo* gene delivery to the liver in both humans and animal models [1]. Often, delivery of the transgene is mediated by an adenoviral vector. Unfortunately, however, infusion of an adenoviral vector into the liver can precipitate dangerous systemic inflammatory responses [2]. Therefore, to minimize adenovirus-related toxicity, efforts have been made to localize gene transfer and expression to specific liver segments. In that regard, physically targeting gene delivery by isolating the hepatic perfusion might be a more promising approach to *in vivo* hepatic gene therapy than transcriptional targeting using a liver-specific promoter [3], as it would minimize systemic exposure to the transgene. Such isolated perfusion of whole liver or selected liver segments has already proven to be useful for studying hepatic metabolism and for administration of cancer chemotherapy, and could be useful for efficient adenovirally or retrovirally mediated gene transfer [4, 5].

In the present study, therefore, we examined the feasibility of physically targeting delivery of a gene encoded in an adenoviral vector to selected hepatic lobes.

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MATERIALS AND METHODS

Animals

All experiments were carried in accordance with the guidelines for the care and use of animals at the Nippon Medical School, Japan. Female Wistar rats (8–10 weeks-old; 170–200 g) were purchased from Nihon CLEA Co., Ltd. (Tokyo, Japan) and maintained under standard laboratory conditions on a 12 h light/12 h darkness cycle and a standard laboratory diet. No food was given for 12 h preceding surgery, but the animals were allowed drinking water.

Viral Vectors

An adenoviral vector expressing the *E. coli* β-galactosidase (β-gal) gene, Adex1CAlacZ, was kindly provided by Izumi Saito of the Institute of Medical Science, University of Tokyo. Because the E1A, E1B, and E3 regions were deleted from the adenoviral type 5 genome, this vector is replication-defective, and the β-gal gene was driven by the CAG (cytomegalovirus enhancer-chicken β-actin hybrid) promoter [6]. The recombinant virus was purified through two cycles of cesium chloride gradient centrifugation and titrated [7]. The viral titer used for these experiments was 3×10^{10} plaque-forming units (pfu)/mL.

Surgical Procedures and *In Vivo* Gene Transfer

All surgical procedures were carried out under nonsterile but clean conditions. Rats were anesthetized with ether, after which 200 U of heparin were administered intravenously to prevent thrombosis. The rats were divided into two groups: the isolated hepatic segment perfusion (IHSP) group received the transgene into the targeted lobes as described below, while the inferior vena cava (IVC) group merely received the transgene directly *via* the IVC without isolation of the hepatic circulation.

The surgery entailed first opening the abdomen through a midline incision, dividing the ligamentous attachments of the liver, and exteriorizing the intestine, which was then wrapped in gauze soaked in warm normal saline. The portal vein was then cannulated with a 29-gauge inflow-catheter *via* the pyloric vein, which is the most proximal tributary of the portal vein. The IVC was cannulated with a 23-gauge outflow-catheter *via* the right iliopsoas vein so that the tip of the IVC catheter was situated under the liver. Sites distal to the cannulations were permanently ligated. For complete vascular isolation of the liver, all normal in- and outflow routes were temporarily clamped: the suprahepatic IVC was closed with a clip between the diaphragm and the liver, the infrahepatic IVC with a ligature between the liver and the right renal vein, the portal vein with a ligature just proximal of the pyloric branch, and the hepatic artery with clip.

Initially, the entire liver was flushed with 4 mL of phosphate buffered saline (PBS) to eliminate all blood and prevent peripheral hepatic embolisms. In addition, the portal vein was clamped between the upper and lower liver segments in order to selectively perfuse the proximal segments of the liver. The targeted liver segments were administered 3×10^9 pfu of adenoviral vector diluted in 1 mL of PBS through the PV catheter and incubated for 5 min. They were then flushed with 8 mL of PBS to wash out any unbound vector particles. All outflow solution was collected from the IVC catheter. After perfusion, the temporary clips and ligatures were removed, as were the PV and IVC catheters, and the cannulated vessels were ligated. The interruption of systemic circulation was less than 15 min. A schematic representation of the IHSP technique and liver lobes are shown in Fig. 1. Using this technique, targeted lobes are RU (right upper, 1.073g), RL (right lower, 0.474g), T (tail, 0.531g), and non-targeted lobes are L (left, 1.907g), ML (middle left, 0.696g;), MR (middle right, 1.233 g).

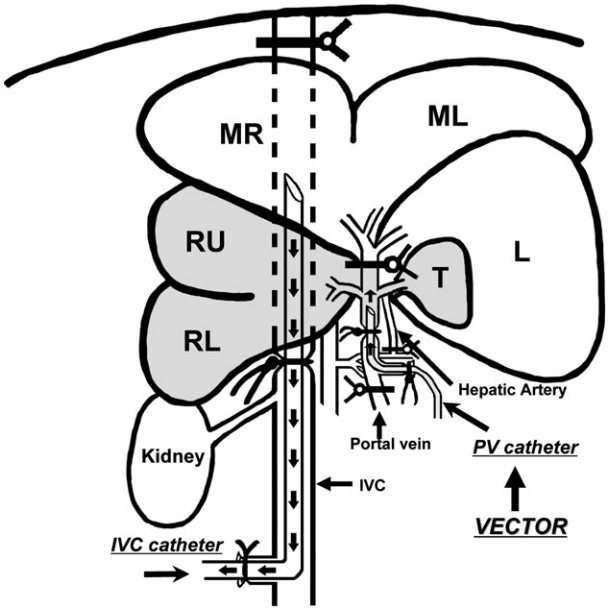

FIG. 1. Schematic representation of the IHSP technique. To perfuse selected lobes of the liver, the portal vein was clamped between the upper (L, ML, MR) and lower (RU, RL, T) segments. For complete vascular isolation of liver, after cannulating the portal vein and IVC, temporary clamps or ligatures were placed on the suprahepatic IVC, infrahepatic IVC, hepatic artery, and portal vein. The targeted liver segments were administered 3×10^9 pfu of adenoviral vector through the PV catheter and incubated for 5 min. All outflow solution was collected from the IVC catheter. After perfusion, the temporary clamps and ligatures were removed, as were the PV catheter and IVC catheter, and the cannulated vessels were ligated. Targeted lobes are RU, RL, and T (shaded). Non-targeted lobes are L, ML, and MR.

X-gal and HE Staining

For histochemical staining, rats were sacrificed 72 h after administration of the adenoviral vector and perfused intravenously with PBS. The liver lobes (L, ML, RU, and RL) and spleens were collected, frozen, and embedded in O.C.T. compound (SAKURA, Tokyo, Japan), after which 5-μm-thick frozen sections were cut on a cryostat and fixed in fixative solution (1% paraformaldehyde, 0.2% glutaraldehyde, 0.02% Nonidet P40) for 30 min at 4°C. The preparations were then immersed in X-gal (5-bromo-4-chloro-indolyl-β-D-galactopyranoside) staining solution (1 mg/mL X-gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM $MgCl_2$) for 3 h at 37°C. After coloration, the sections were stained with HE.

β-gal Activity

To assay β-gal activity, rats were sacrificed 72 h after administration the adenoviral vector and perfused intravenously with PBS. The liver lobes (L, ML, MR, RU, RL, and T), spleens, kidneys, lungs, and hearts were then collected and homogenized separately in lysis buffer containing 40 mM Tris, 140 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (a protease inhibitor), and 1 mM dithiothreitol. After clearing, the homogenates by centrifugation at 10,000 $\times$ g for 10 min at 4°C, the supernatants were collected, and diluted with lysis buffer to a final volume of 150 μL. A 150 μL aliquot of assay buffer containing 120 mM dibasic sodium phosphate, 80 mM sodium biphosphate, 2 mM $MgCl_2$, 100 mM 2-mercaptoethanol, and 1.33 mg/ml O-nitrophenyl-β-D-galactopyraniside (ONPG) was added to each supernatant and incubated for 30 min at room temperature. The reaction was then stopped by adding 500 μL of a 2.8% sodium carbonate solution. The β-gal activity was quantified based on the cleavage of