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Liver- and lobe-selective gene transfection following the instillation of plasmid DNA to the liver surface in mice

Shigeru Kawakami,[a,*] Ryu Hirayama,[a] Keiko Shoji,[a] Rie Kawanami,[a] Koyo Nishida,[a] Mikiro Nakashima,[b] Hitoshi Sasaki,[b] Toshiyuki Sakaeda,[c] and Junzo Nakamura[a]

[a] *School of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan*
[b] *Department of Hospital Pharmacy, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan*
[c] *Department of Hospital Pharmacy, School of Medicine, Kobe University, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan*

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Abstract

The present study has undertaken the liver- and lobe-selective gene transfections following the instillation of plasmid DNA (pDNA) to the liver surface in mice. The luciferase levels produced in the applied (left) liver lobe at 6 h after liver surface instillation of pDNA were significantly higher than those produced in the other tissues assayed, and ranged from 8.5-fold higher in other liver lobes to 320-fold higher in other tissues. After small intestine surface instillation of pDNA, the gene expression was a little detected in the tissues assayed. Following liver surface instillation of pDNA at a time from 2 to 48 h or at a volume from 15 to 120 μl, the gene expressions of the applied liver lobe were always significantly higher than those of other liver lobes and other tissues. We demonstrated the novel liver- and lobe-selective gene transfection utilizing the instillation to the liver surface.

Keywords: Gene therapy; Liver; Plasmid DNA; Transfection; Mice; In vivo

Gene therapy is a novel therapeutic method for the treatment of refractory diseases such as tumors [1–3]. The most important factor for successful gene therapy is the development of novel gene transfection vectors, therefore, various viral vectors or non-viral vectors were studied. When the genes were administered by the vasculature route, they were distributed to the whole body via the blood stream, leading to inadequate organ-selective and diseased site-selective gene delivery. Although, it was reported that the direct injection of genes to the organ should yield the organ-selective and diseased site-selective gene transfection [3–5], there is great concern about safety because of injury to organs by the needles and the administration volume limits, etc. Previously, we developed the application of drugs to the liver surface and found it be a useful method for site-selective drug delivery to the liver [6–9]. The liver- and lobe-selective gene targeting strategy has the potential for wide clinical applications and is a safe and convenient method for the in vivo delivery of therapeutic proteins. The present study was undertaken to elucidate the liver- and lobe-selective gene transfection following the instillation of plasmid DNA (pDNA) to the liver surface in mice.

Materials and methods

Materials. All chemicals were of the highest purity available.

Construction and preparation of pDNA (pCMV-Luc). pCMV-Luc was constructed by subcloning the *Hin*dIII/*Xba*I firefly luciferase cDNA fragment from pGL3-control vector (Promega, Madison, WI, USA) into the polylinker of pcDNA3 vector (Invitrogen, Carlsbad, CA, USA). pDNA was amplified in the *Escherichia coli* strain DH5α, isolated, and purified using a QIAGEN Plasmid Giga Kit (QIAGEN GmbH, Hilden, Germany).

In vivo gene expression experiments. All animal procedures in the present study conformed to the Guidelines for Animal Experimentation in Nagasaki University. Five-week-old ddY male mice (20.0–32.0 g) were anesthetized with sodium pentobarbitone (40–60 mg/kg). The middle abdomen was cut open and pDNA (15–120 μl) at a dose of 30 μg was administered intraperitoneally to either the surface of the

* Corresponding author. Fax: +81-95-845-7218.
E-mail address: kawakami@net.nagasaki-u.ac.jp (S. Kawakami).

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liver or the small intestine using micropipette (PIPETMAN, GILSON, Villier-le Bel, France). The instillation point of pDNA at the surface of the liver and small intestine was the left liver lobe and about 2 cm below the instillation point at the liver surface, respectively. At 1 min, the abdomen was sutured. Mice were kept lying on their back for 1 h, then they were freed in the cage. At appropriate time intervals, mice were sacrificed, and the liver, kidney, spleen, heart, and lung were removed. Then, the applied (left) liver lobe was separated from other liver lobes. The tissues were washed twice with saline and homogenized with a lysis buffer. The lysis buffer consisted of 0.1 M Tris/HCl buffer (pH 7.8) containing 0.05% Triton X-100 and 2 mM EDTA [10]. The volumes of the lysis buffer added were 4 μl/mg for each liver lobe and 5 μl/mg for other tissues. After three cycles of freezing and thawing, the homogenates were centrifuged at 15,610*g* for 5 min. The supernatants were stored at −20 °C until the luciferase assays were performed. Twenty microliters of supernatant was mixed with 100 μl luciferase assay buffer (Picagene, Toyo Ink, Tokyo, Japan) and the light produced was immediately measured using a luminometer (Lumat LB 9506, EG & G Berthold, Bad Wildbad, Germany). The luciferase activity is indicated as the relative number of light units per gram of tissue.

Statistical analysis. Statistical comparisons were performed by analysis of variance. $P < 0.05$ was considered to be indicative of statistical significance.

Results and discussion

Fig. 1 shows the gene expression in the liver, kidney, spleen, heart, and lung at 6 h after liver surface instillation, small intestine surface instillation, and intravenous administration of pDNA at a dose of 30 μg into mice. The gene expression levels represent more than 2×10^3 RLU/g tissues because each tissue mixed with substrates without the instillation of pDNA showed approximately 2×10^3 RLU/g tissues. Therefore, the results represent greater than 10^4 RLU/g tissues in Fig. 1, which was considered as stable gene expression. After intravenous administration of pDNA, the gene expression was little detected in all tissues assayed except for the kidney. This result is in agreement with previous findings [11]. The luciferase levels produced in the applied (left) liver lobe after liver surface instillation of pDNA were significantly higher than those produced in other tissues assayed, and ranged from 8.5-fold higher in other liver lobes to 320-fold higher in other tissues. After small intestine surface instillation of pDNA, the gene expression was detected in small amounts in all tissues assayed. This result agrees with those of a previous study on the gene expressions after intraperitoneal administration of pDNA [12]. Thus, the present results suggested that the liver surface instillation of pDNA as a novel administration method is effective for liver- and lobe-selective gene transfections. Fig. 2 shows the time course of gene expression in the liver, kidney, spleen, and lung until 48 h after liver surface instillation of pDNA at a dose of 30 μg into mice. The gene expressions of the applied liver lobe from 2 to 48 h were always significantly higher than those of other liver lobes and other tissues. The highest gene expression in the applied

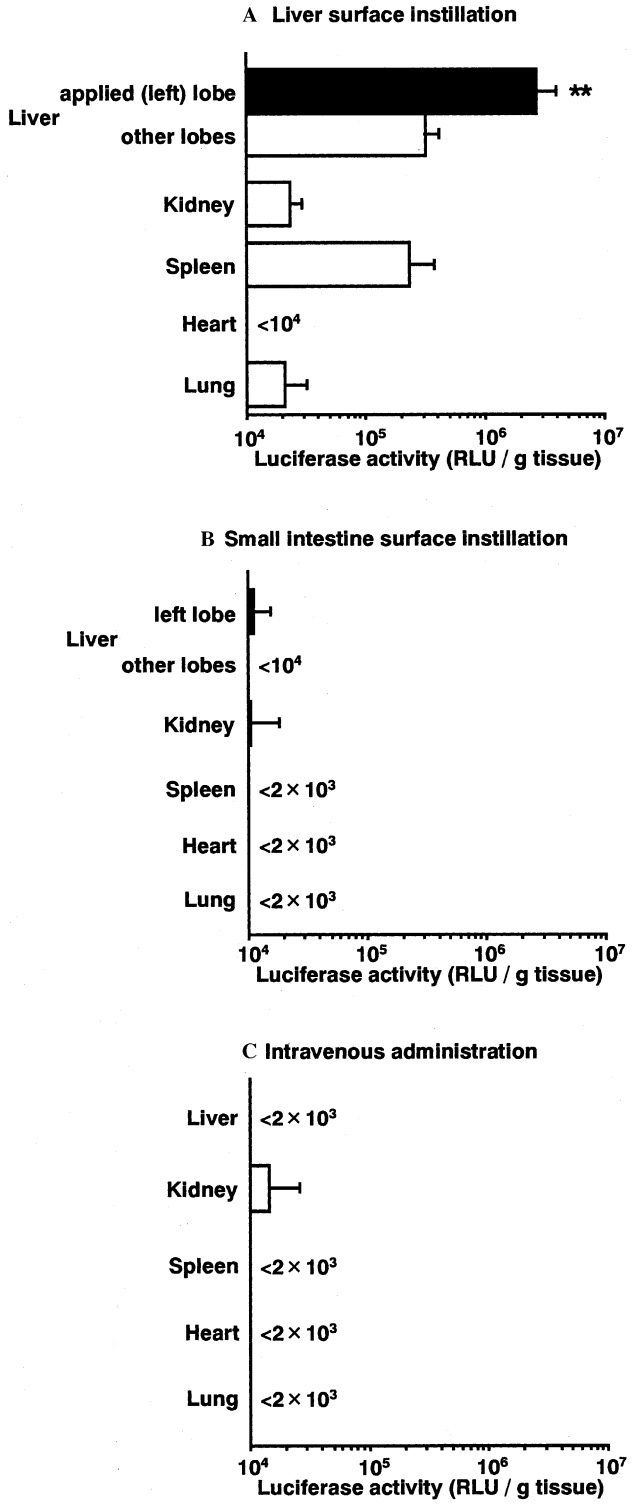

Fig. 1. The gene expression in the liver, kidney, spleen, heart, and lung at 6 h after liver surface instillation (A), small intestine surface instillation (B), and intravenous administration (C) of pDNA at a dose of 30 μg (30 μl) into mice. Statistical comparisons were performed by analysis of variance (**$P < 0.01$ vs. other liver lobes and tissues). Each value represents the mean ± SE of at least eight experiments.

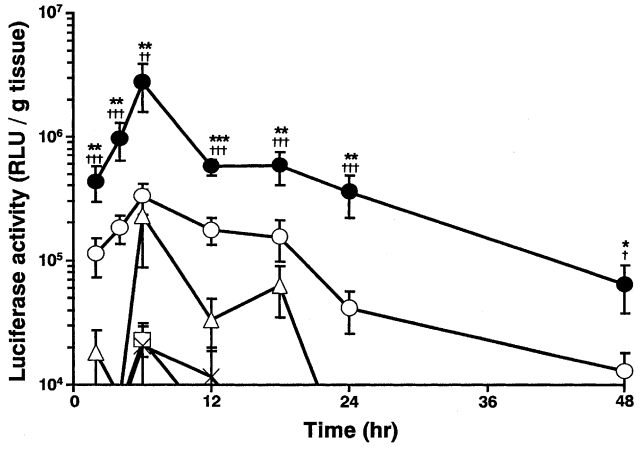

Fig. 2. The time course of gene expression in the applied liver lobe (●), other liver lobes (○), kidney (□), spleen (△), and lung (×) until 48 h after liver surface instillation of pDNA at a dose of 30 μg (30 μl) into mice. Statistical comparisons were performed by analysis of variance ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. other liver lobes; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. other tissues). Each value represents the mean ± SE of at least nine experiments.

liver lobe was observed at 6 h and was gradually diminished thereafter. Fig. 3 shows the effect of the instillation doses of pDNA on gene expression in the liver, kidney, spleen, and lung at 6 h after liver surface instillation of pDNA at doses of 10, 30, and 50 μg into mice. The gene expressions in the applied liver lobe at doses of 10, 30, and 50 μg were always significantly higher than those of the other liver lobes and other tissues. Fig. 4 shows the effect of the instillation volumes of pDNA on gene expression in the liver, kidney, spleen, heart, and lung at 6 h after liver surface instillation of pDNA at a

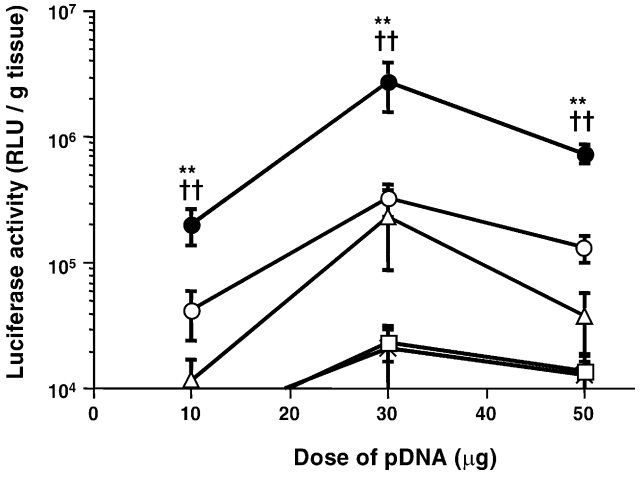

Fig. 3. The effect of instillation doses on gene expression in the applied liver lobe (●), other liver lobes (○), kidney (□), spleen (△), and lung (×) at 6 h after liver surface instillation of pDNA at doses of 10, 30, and 50 μg (30 μl) into mice. Statistical comparisons were performed by analysis of variance ($^{**}P < 0.01$ vs. other liver lobes; $^{\dagger\dagger}P < 0.01$ vs. other tissues). Each value represents the mean ± SE of at least eight experiments.

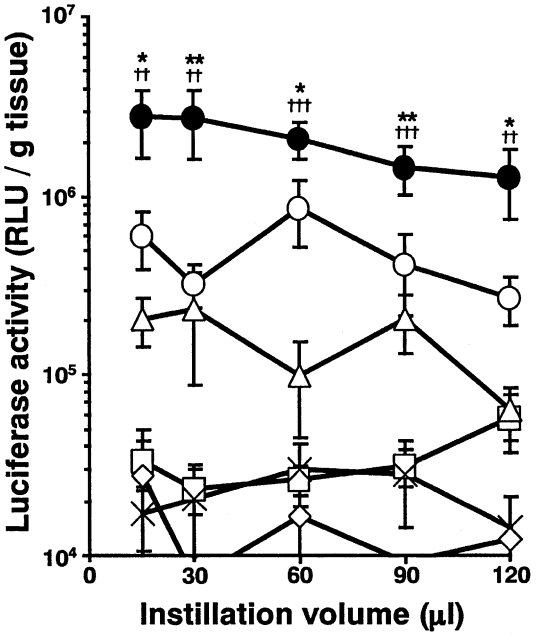

Fig. 4. The effect of instillation volumes on gene expression in the applied liver lobe (●), other liver lobes (○), kidney (□), spleen (△), heart (◇), and lung (×) at 6 h after liver surface instillation of pDNA at a dose of 30 μg into mice. Statistical comparisons were performed by analysis of variance ($^{*}P < 0.05$, $^{**}P < 0.01$ vs. other liver lobes; $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. other tissues). Each value represents the mean ± SE of at least nine experiments.

dose of 30 μg into mice. Although the gene expressions in the applied liver lobe at a volume from 15 to 120 μl were always significantly higher than those of the other liver lobes and other tissues, the gene expression levels in the applied liver lobe were gradually diminished with increasing instillation volume. This may be due to the spread of the pDNA solution from applied liver lobe.

Gene transfer to hepatocytes should be of great therapeutic potential since hepatocytes are responsible for the synthesis of a wide variety of proteins, which play important physiological roles. There has been much interest in in vivo gene transfer to the liver, as an alternate to ex vivo methods, which require invasive surgery [13]. It is difficult to achieve an efficient transfection in vivo. Consequently, various viral and non-viral gene transfection vectors were developed to enhance the transfection efficiency [14]. However, many important technological problems remain. Despite the high transfection efficiency of viral vectors, safety concerns have been raised in clinical trials because of their highly toxic nature. As a result, the use of non-viral vectors has attracted great interest for in vivo gene delivery because they lack some of the risks inherent in viral vector systems. However, the transfection efficiency in the liver is very low despite their high uptake by the liver after intravenous injection of pDNA [15,16]. Kawabata et al. [15] reported that ^{32}P-labeled pDNA ([^{32}P]pDNA) is rapidly eliminated from the plasma involving extensive

uptake by the liver after intravenous injection in mice. Pharmacokinetic analysis has demonstrated that the hepatic uptake clearance of [^{32}P]pDNA is almost identical to the plasma flow rate in the liver, suggesting highly effective elimination by this organ. In addition, [^{32}P]pDNA is taken up preferentially by non-parenchymal cells (macrophages) in liver via a scavenger receptor-mediated process. These findings suggest that plasmid DNA is taken up by macrophages via a mechanism mediated by scavenger receptor. Thus, when pDNA was administered by a vasculature route, they were deprived of the transfection ability by the degradation in Kupffer cells.

In recent years, greater than 50% of all current clinical gene therapy trials such as immune gene therapy and gene-directed enzyme prodrug therapy have been for cancer treatment [3]. The most important factor for gene therapy is an efficient in vivo gene transfection methodology. It was reported that cationic liposome-mediated gene transfection is one of the promising approaches due to the transfection efficiency [17–21]. In most cases, however, the highest gene expression in the lung is observed after intravenous injection of cationic liposome/pDNA complexes because the lung capillaries are the first ''traps'' to be encountered [21]. Receptor-mediated gene targeting appears to be a promising approach to obtain the hepatic organ (or cell)-selective gene transfection. A number of receptor-mediated gene delivery systems have been developed to introduce the foreign DNA into specific cell types [10,22–26]. Among the various receptors, asialoglycoprotein receptors [10,22–24] and mannose receptors [25,26] are the most promising for hepatic gene targeting in vivo since they exhibit high affinity and are rapidly internalized. However, its cell selectivity depended on the physicochemical properties of the pDNA complexed with glycosylated cationic liposomes and/or cationic polymers [10,25]. The interaction between the pDNA/cationic carrier complexes and other organs or erythrocytes via the electrostatic interaction after intravenous administration also leads to decreased transfection activity at the targeted cells. In addition, it is difficult to achieve the liver site-selective gene transfection using pDNA/glycosylated cationic liposome complexes after intravenous administration or intraportal administration [25] because they were distributed to the whole liver (or body) via the blood stream.

In summary, we have demonstrated here, for the first time, the liver- and lobe-selective gene transfection system utilizing the instillation of pDNA to the liver surface in mice. The transfection levels of the applied liver lobe were significantly higher than that of other liver lobes (Figs. 1–4). The liver- and lobe-selective gene transfection method is expected to be a safe and effective treatment against hepatic cancer, which is localized in part of the liver. The liver surface instillation of pDNA is not stressful against the liver, so that continuous administration of pDNA is possible by use of a catheter to enable long-term hepatic gene transfection. Furthermore, these results suggested that this novel gene administration method could be applied to other organ- and site-selective gene transfections. For the possibility of the absorption of pDNA from the liver surface and other organ- and site-selective gene transfections utilizing organ surface instillations of pDNA, additional studies are in progress.

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