Significant Role of Liver Sinusoidal Endothelial Cells in Hepatic Uptake and Degradation of Naked Plasmid DNA After Intravenous Injection

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Received October 15, 2003; accepted March 14, 2004

Purpose. Uptake and degradation of naked plasmid DNA (pDNA) by liver sinusoidal endothelial cells (LSECs) were investigated.
Methods. Tissue distribution and intrahepatic localization were determined after an intravenous injection of ^{111}In- or ^{32}P-labeled pDNA into rats. Cellular uptake and degradation of fluorescein- or ^{32}P-labeled pDNA were evaluated using primary cultures of rat LSECs.
Results. Following intravenous injection, pDNA was rapidly eliminated from the circulation and taken up by the liver. Fractionation of liver-constituting cells by centrifugal elutriation revealed a major contribution of LSECs to the overall hepatic uptake of pDNA. Confocal microscopic study confirmed intracellular uptake of pDNA in cultured LSECs. Apparent cellular association of pDNA was similar at 37°C and 4°C. However, trichloroacetic acid (TCA) precipitation experiments showed the TCA-soluble radioactivity in the culture medium increased in an accumulative manner at 37°C. Involvement of a specific mechanism was demonstrated, as the uptake of pDNA was significantly inhibited by excess unlabeled pDNA and some polyanions (polyinosinic acid, dextran sulfate, heparin) but not by others (polycytidylic acid, dextran). These inhibitors also reduced the amount of TCA-soluble radioactivity in the culture medium.
Conclusion. These results suggest that LSECs efficiently ingested and rapidly degraded naked pDNA *in vivo* and *in vitro* and released the degradation products into the extracellular space.

KEY WORDS: degradation; liver sinusoidal endothelial cells; plasmid DNA; trichloroacetic acid; uptake.

INTRODUCTION

Plasmid DNA (pDNA), the simplest nonviral vector, is an important macromolecular agent for gene therapy or DNA vaccination (1,2). Though relatively low transfection efficiency is one of the main limiting factors of nonviral gene transfer strategies, pDNA has advantages in terms of its safety and versatility compared with viral vectors that potentially exhibit immunogenicity and undergo mutation thereby reacquiring the ability to produce infection.

In our previous study involving the *in vivo* disposition of pDNA, we demonstrated that pDNA is rapidly removed from the circulation, more quickly than its degradation by nucleases in the blood and other compartments, and taken up by the liver, predominantly by the liver nonparenchymal cells, after intravenous administration to mice (3). We also demonstrated the key role played by liver nonparenchymal cells in the hepatic uptake of pDNA in the single-pass rat liver perfusion system (4). In addition, we found that the hepatic uptake of pDNA involves a specific mechanism similar to, but distinct from, a class A scavenger receptor (SRA), as it was dramatically inhibited by specific polyanions such as polyinosinic acid (poly I) and dextran sulfate but not by polycytidylic acid (poly C) in mice as well as in a rat liver perfusion system; in addition, it was not significantly affected in SRA-knockout mice (3–5). More recently, we suggested that, among the nonparenchymal cells, liver sinusoidal endothelial cells (LSECs), rather than Kupffer cells, made a major contribution to the overall uptake of pDNA, as gadolinium chloride-induced blockade of Kupffer cells did not affect the degree of hepatic uptake of [^{32}P]pDNA in mice (6). However, there has been no direct evidence for the large contribution of LSECs to the hepatic uptake of pDNA.

LSECs are known to play an important role in the induction of immune tolerance (7,8). In addition, the potential capability of LSECs as a major scavenger of circulating DNAs gives rise to the possibility that LSECs participate in the onset of systemic lupus erythematosus, a disease characterized by the production of anti-DNA antibody (9). In order to develop a strategy for optimizing pDNA delivery in gene therapy and DNA vaccination, it is important to elucidate the types of liver cells involved in pDNA uptake because this might be related to pDNA-derived gene expression and pDNA-induced immune responses. In the current study, therefore, we investigated quantitatively the contribution of LSECs to the hepatic uptake of pDNA following intravenous injection in the rat and studied more details of the cellular uptake characteristics of pDNA *in vitro* using primary cultures of rat LSECs.

MATERIALS AND METHODS

Chemicals

[α-^{32}P]dCTP (3000 Ci/mmol) and dextran (MW 70,000) were obtained from Amersham (Buckinghamshire, England). ^{111}Indium chloride was supplied by Nihon Medi-Physics Co. (Takarazuka, Japan). Poly I (MW 103,300) and poly C (MW 99,500) were purchased from Pharmacia (Uppsala, Sweden). Dextran sulfate (MW 150,000) and heparin sodium salt were purchased from Nacalai Tesque (Kyoto, Japan). Type I-A collagenase, dexamethasone, and vascular endothelial growth factor (VEGF) were purchased from Sigma (St. Louis, MO, USA). Type I rat tail collagen and ITS(+) were purchased from BD Biosciences (San Jose, CA, USA). All other chemicals used were of the highest purity available.

Plasmid DNA

pCMV-Luc (10) encoding firefly luciferase was used as a model pDNA throughout the current study. The pDNA amplified in the DH5α strain of *Escherichia coli* was extracted and purified by a QIAGEN Endofree Plasmid Giga Kit (QIAGEN GmvH, Hilden, Germany). The purity was checked by 1% agarose gel electrophoresis followed by ethid-

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ABBREVIATIONS: pDNA, plasmid DNA; poly I, polyinosinic acid; poly C, polycytidylic acid; LSECs, liver sinusoidal endothelial cells; SRA, class A scavenger receptor; TCA, trichloroacetic acid.

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ium bromide staining. The pDNA concentration was measured by UV absorption at 260 nm. For biodistribution or cellular uptake studies, pDNA was radiolabeled with ^{111}In as described below or with [α-^{32}P]dCTP by the nick translation method (11). For confocal microscopic observations, pDNA was labeled with fluorescein using a FastTag FL labeling kit (Vector Laboratories, Burlingame, CA, USA).

Preparation of [^{111}In]pDNA

Radiolabeling of pDNA with ^{111}In was performed by a method described elsewhere (12). Briefly, to a 27.5-μl dimethylsulfoxide solution of 1 mg 4-[*p*-azidosalicylamido]butylamine (ASBA) was added diethylenetriaminepentaacetic acid (DTPA) anhydride (2 mg) under dark-room conditions, and the mixture was incubated at room temperature for 1 h Then, 25 μl pDNA solution (4 mg/ml) was added to the mixture, and the volume was adjusted to 500 μl with phosphate-buffered saline (PBS) of pH 7.4. The mixture was immediately irradiated under a UV lamp (365 nm, UltraViolet Products, Upland, CA, USA) at room temperature for 15 min to obtain DTPA-ASBA coupled pDNA (DTPA-ASBA-pDNA). The product was purified by ethanol precipitation twice and was dissolved in 20 μl acetate buffer (0.1 M, pH 6). To 10 μl sodium acetate solution (1 M) was added 10 μl $^{111}InCl_3$, then 20 μl DTPA-ASBA-pDNA. The mixture was incubated at room temperature for 1 h, and unreacted $^{111}InCl_3$ was removed by ultrafiltration. The purity was checked by Sephadex G-25 column (1 × 40 cm) chromatography and 1% agarose gel electrophoresis.

In Vivo Disposition Studies of pDNA

Male Wistar rats (250 g) were purchased from the Shizuoka Agricultural Co-operate Association for Laboratory Animals (Shizuoka, Japan). All animal experiments were reviewed and approved by the Ethics Committee for Animal Experiments at the Kyoto University.

Rats received [^{32}P]pDNA or [^{111}In]pDNA diluted with unlabeled pDNA (1 mg/kg) in sterilized saline by tail vein injection. The tail vein injection was performed using a 26-gauge needle. Blood was withdrawn from the jugular vein at the indicated times following pDNA injection under anesthesia and then centrifuged to obtain plasma. The rats were euthanized 30 min after urine collection from the urinary bladder, and then the kidney, liver, lung, and heart were excised and rinsed with saline. In the case of [^{32}P]pDNA, the samples of plasma, urine, and small pieces of tissue were dissolved in 0.7 ml Solene-350 at 45°C, followed by the addition of 0.2 ml isopropanol, 0.2 ml H_2O_2, 0.1 ml 5N HCl, and 5 ml Clearsol I (scintillation medium). The radioactivity was measured using a liquid scintillation counter (LSA-500, Beckman, Tokyo, Japan). In the case of [^{111}In]pDNA, the radioactivity of the samples was directly measured in a NaI scintillation counter (ARC-500, Aloka, Tokyo, Japan).

To investigate the intrahepatic distribution of pDNA, rats received [^{111}In]pDNA (1 mg/kg) by tail vein injection under anesthesia. The rats were killed at 30 min, and then the liver was fractionated into parenchymal cells, Kupffer cells, and LSECs as mentioned below. The radioactivity of each cell suspension was measured in a NaI scintillation counter.

Isolation and Culture of Primary LSECs

Isolation of LSECs was performed as previously described (13). Briefly, liver was perfused under anesthesia via the portal vein with Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (HBSS) at 37°C for 10 min at a flow rate of 10–12 ml/min. Then, the liver was perfused with HBSS containing 5 mM Ca^{2+} and 0.05% (w/v) collagenase for 10 min. The digested liver was minced and filtered through a cotton gauze and a nylon mesh (mesh size 45 μm) and then fractionated into hepatocytes and nonparenchymal cells by differential centrifugation. Liver nonparenchymal cells were further separated into LSECs and Kupffer cells by centrifugal elutriation. LSECs were seeded on 24-well plates (1.0 × 10^6 cells/well), coated beforehand with rat tail collagen, and cultured in RPMI1640 supplemented with 10% fetal calf serum, VEGF (5 μg/ml), ITS(+) (1% v/v), amphotericin B (10 mg/ml), and dexamethasone (0.01 mM) for 4–5 days. The purity of LSECs was checked by immunostaining of factor VIII–related antigen as well as uptake experiment using microparticulates (4.5 μm) and confirmed to be more than 95%. We also ensured the expression of functional receptors on the isolated LSECs in our preliminary *in vitro* uptake experiment using mannosylated BSA (data not shown).

In Vitro Cellular Association Experiments

LSECs were washed three times with 0.5 ml HBSS followed by the addition of 0.5 ml HBSS containing 0.1 μg/ml naked [^{32}P]pDNA. After incubation at 37°C or 4°C for a specified time, incubation medium was removed and the cells were washed five times with ice-cold HBSS and then solubilized with 1.0 ml of 0.3 N NaOH containing 0.1% Triton X-100. Aliquots of the cell lysate were subjected to the determination of ^{32}P radioactivity. To examine the competitive effects of various polyanions on the binding and uptake of pDNA, the described dose of unlabeled pDNA or macromolecules such as poly I, poly C, and dextran sulfate was added to the incubation medium concomitantly with [^{32}P]pDNA.

TCA Precipitation Experiments

To estimate the amount of degradation products of pDNA, following completion of the cellular association experiments, the culture medium and cell lysates were subjected to trichloroacetic acid (TCA) precipitation. After elimination of cellular proteins by extracting with TE buffer-saturated phenol (69% phenol), aliquots of the supernatants and cell lysates were mixed with TCA to give a final concentration of 5% (w/v), kept on ice for 10 min, and then centrifuged at 13,000 rpm for 30 min. The supernatant (TCA-soluble fraction) was subjected to radioactivity measurement. The TCA-soluble fraction is supposed to contain small DNA fragments of degradation products (short oligonucleotides), as longer oligonucleotides (>16-mer) are precipitable in 5% TCA (14).

Confocal Microscopic Study

LSECs cultured on the cover glasses were washed with HBSS and mixed with 5 μg/ml fluorescein-labeled pDNA ([FL]pDNA). After incubation at 37°C or 4°C, the cells were washed and fixed with 4% paraformaldehyde for 1 h. Then, cell sheets were stained with an anti-factor VIII–related an-

tigen mouse IgG (InnoGenex, San Ramon, CA, USA) at 1:200 dilution followed by secondary antibodies, an anti-mouse IgG Alexa Fluor 594 (Molecular Probes, Eugene, OR, USA) at 1:250 dilution. Finally, these samples were subjected to confocal microscopic investigation (MRC-1024, Bio-Rad, Hercules, CA, USA).

RESULTS

In Vivo Disposition of pDNA After Intravenous Injection

First, we examined the *in vivo* disposition of pDNA in rats. Figure 1 shows the time-courses of the plasma concentration of radioactivity and the amounts of radioactivity in the organs and urine at 30 min after intravenous injection of [^{32}P]pDNA or [^{111}In]pDNA (1 mg/kg). pDNA was rapidly eliminated from the circulation and mainly taken up by the liver, similarly to previous studies using mice (3,6). No significant difference was observed, at least during this experimental period, between the radiolabeling methods in terms of the pattern of elimination from plasma and the degree of liver accumulation.

To examine the contribution of liver-constituting cells to the hepatic uptake of pDNA, hepatocytes, LSECs, and Kupffer cells were isolated from the liver following intravenous administration of pDNA. Figure 2 shows the intrahepatic distribution of pDNA at 30 min after intravenous injection of [^{111}In]pDNA at a dose of 1 mg/kg. The amount of pDNA taken up by LSECs and Kupffer cells on a cellular basis (i.e., per 10^8 cells) were significantly greater than that by hepatocytes (Fig. 2A). The relative contributions of hepatocytes, Kupffer cells and LSECs were evaluated on the basis of the numbers of each cell per liver (15,16). As shown in Fig. 2B, pDNA was mostly taken up by LSECs (almost 50% of total hepatic uptake). The estimated total hepatic recovery of injected radioactivity, which was calculated from the data in Fig. 2A on the basis of the number of each cell per 1 g liver assuming the weight of the rat liver to be 8 g, was approximately 70% of the dose, in agreement with the direct measurement of the hepatic accumulation of [^{111}In]pDNA (Fig. 1B). However, in our preliminary study using [^{32}P]pDNA, the estimated liver accumulation following isolation of liver-constituting cells was less than 15% of the dose (data not shown), which was much lower than that determined in Fig. 1B.

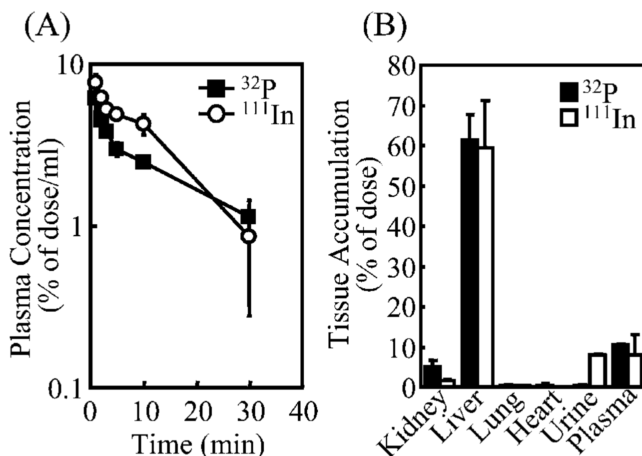

Fig. 1. Time-courses of (A) plasma concentration and (B) tissue accumulation of radioactivity at 30 min after intravenous injection of [^{32}P]pDNA or [^{111}In]pDNA (1 mg/kg) into rats. The results are expressed as mean ± SD (n = 3).

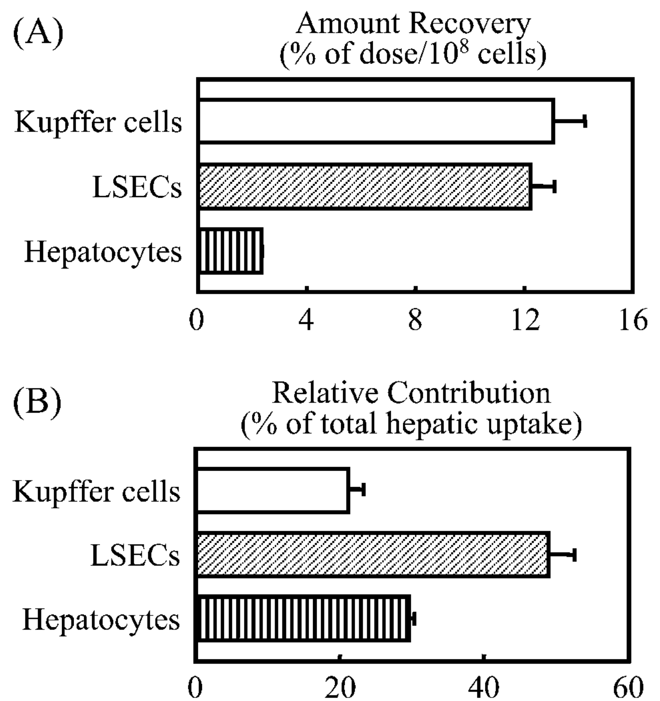

Fig. 2. Intrahepatic distribution of [^{111}In]pDNA (1 mg/kg) on a (A) cellular basis and (B) the relative contribution of each type of liver cell to the total hepatic uptake. Rats were euthanized 30 min after intravenous injection and liver cells were isolated as described in "Materials and Methods." The results are expressed as mean ± SD (n = 3).

Cellular Uptake and Degradation of pDNA in Primary Culture of Rat LSECs

To evaluate the details of the cellular uptake of pDNA by LSECs, *in vitro* studies were performed with primary cultures of rat LSECs. Figure 3 shows the uptake of [FL]pDNA in primary cultures of LSECs assessed by confocal laser scanning microscopy. Fluorescence derived from [FL]pDNA was

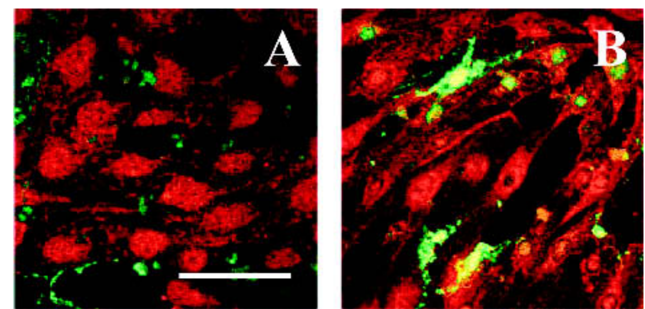

Fig. 3. Confocal microscopic images of LSECs following incubation with [FL]pDNA. The cells were incubated with 0.1 μg/ml [FL]pDNA (green) for 3 h at (A) 4°C or (B) 37°C and fixed with 4% paraformaldehyde and immunostained with anti-factor VIII–related antigen antibody (red). The images shown are typical of those observed in several visual fields. Scale bar represents 50 μm.

restricted to the cell surface or extracellular compartment at 4°C, whereas an intense fluorescence was observed in the intracellular compartment at 37°C.

Figure 4 shows the time-courses of the cellular association and degradation of [^{32}P]pDNA in primary cultures of rat LSECs. The cellular association at 37°C reached a maximum at 1.5 h and then gradually decreased probably due to the release of degradation products into the culture medium. However, at 4°C, a steady increase in the apparent cellular association was observed, although the cells did not appear to internalize pDNA as shown by confocal microscopy. To evaluate the degradation of [^{32}P]pDNA after uptake by LSECs, TCA precipitation experiments were performed. The degradation products of [^{32}P]pDNA in the cellular fraction were at most 2% of the applied dose at 37°C and 4°C (Fig. 4B). On the other hand, a time-dependent dramatic increase was observed in the amount of the degradation products of [^{32}P]pDNA in the culture medium at 37°C, but not at 4°C (Fig. 4C).

Effect of Various Polyanions on Cellular Uptake and Degradation of pDNA in LSECs

We performed competitive studies to examine if the cellular uptake characteristics of pDNA in LSECs were similar to that demonstrated *in vivo* in mice. Figure 5 shows the competitive effects of various macromolecules on the cellular association of [^{32}P]pDNA with LSECs. Excess amounts of unlabeled pDNA inhibited cellular association of [^{32}P]pDNA at 37°C and 4°C. The cellular association was also significantly inhibited by the presence of poly I, dextran sulfate, or heparin, but not by poly C or dextran. To examine further the effect of polyanions on the degradation of [^{32}P]pDNA, the TCA-soluble radioactivity in the culture medium was measured following competitive experiments at 37°C (Fig. 6). Degradation of pDNA was prevented by an excess of unlabeled pDNA and polyanions such as poly I and heparin, which inhibited the cellular association of pDNA.

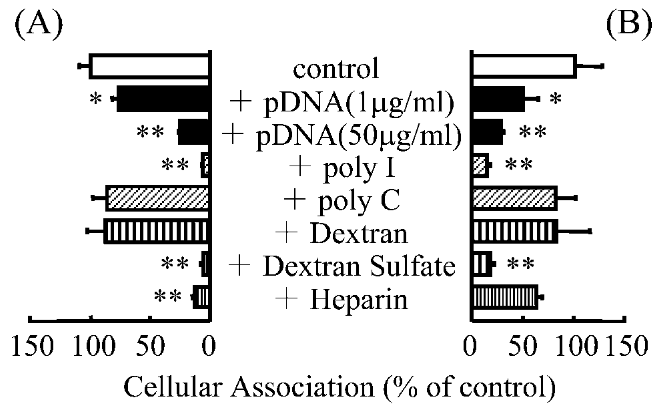

Fig. 5. Competitive effects of various macromolecules on cellular association of [^{32}P]pDNA with LSECs at (A) 4°C or (B) 37°C. The cells were incubated with [^{32}P]pDNA (0.1 μg/ml) for 3 h in the presence or absence of the indicated dose of unlabeled pDNA and various macromolecules (50 μg/ml). The results are expressed as mean ± SD ($n = 3$). Statistical significance was analyzed by Dunnett's test; $^{**}p < 0.01$, $^{*}p < 0.05$ vs. control.

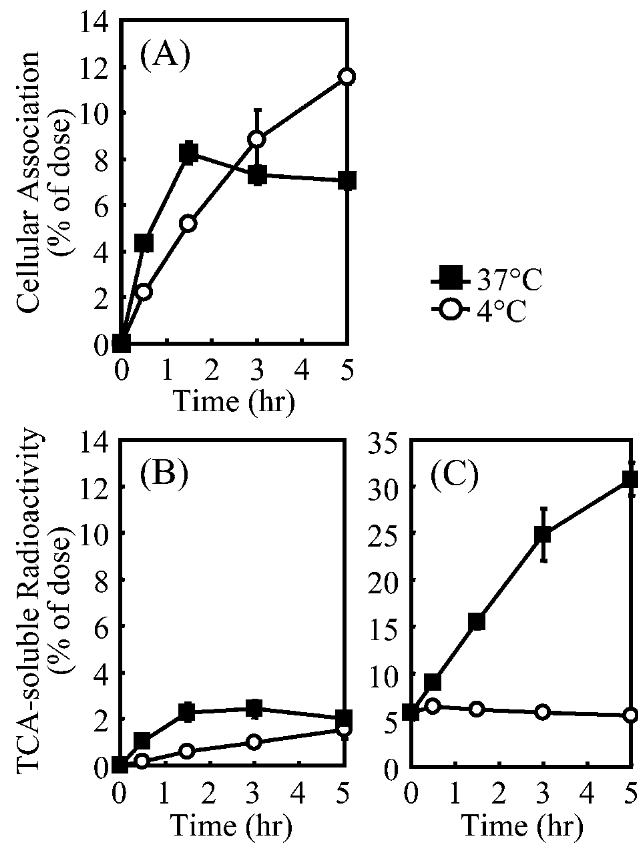

Fig. 4. Time-courses of cellular association of [^{32}P]pDNA with (A) LSECs and TCA-soluble radioactivity in (B) the LSEC cellular fraction and (C) in culture medium. The cells were incubated with [^{32}P]p-DNA (0.1 μg/ml) at 37°C (closed square) or 4°C (open circle). Each point represents the mean ± SD ($n = 3$). The SD was included in the symbol when it was very small.

DISCUSSION

A number of studies involving *in vivo* disposition following intravenous injection of naked DNAs and their complexes, such as single-stranded DNA, double-stranded DNA, oligonucleotide, DNA anti-DNA immune complex, or mononucleosome, have been already reported (17–20). These studies have shown that the liver is the main organ responsible for the rapid clearance of these DNAs from the circulation, while

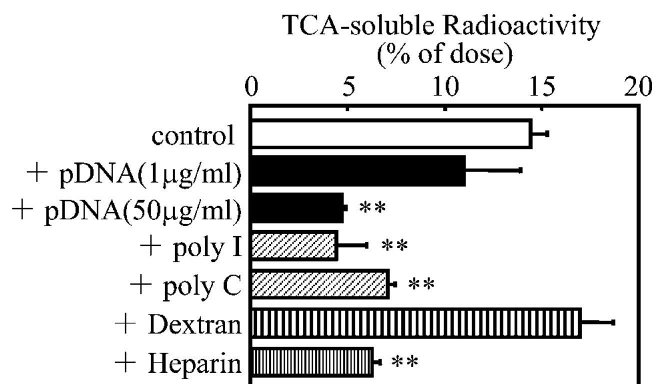

Fig. 6. TCA-soluble radioactivity in the culture medium following competitive experiments at 37°C. The results are expressed as mean ± SD ($n = 3$). Statistical significance was analyzed by Dunnett's test; $^{**}p < 0.01$ vs. control.

the uptake mechanism and the cell-type(s) contributing to this hepatic uptake remain to be elucidated. We have demonstrated predominant uptake of pDNA by liver nonparenchymal cells (3,4) and suggested the relative importance of LSECs for the overall hepatic uptake of pDNA (6). However, due to lack of direct evidence, more detailed studies need to be performed to confirm the significant contribution of LSECs to pDNA hepatic uptake.

In the current study, we demonstrated efficient uptake and degradation of pDNA by LSECs *in vitro* in primary cell cultures and *in vivo* in rats. We used selective radiolabeling strategies, which allowed us to perform a quantitative analysis of pDNA uptake and degradation. Following intracellular degradation of [^{111}In]pDNA following cellular uptake, ^{111}In should be present as a form of ^{111}In-DTPA-ASBA covalently linked to a single nucleotide or oligonucleotides. Because these metabolites are unlikely to cross biological membranes due to their relatively large size and high hydrophilicity, the ^{111}In-radioactivity could remain within the cells, as is the case of ^{111}In-DTPA-lysine for ^{111}In-labeled protein (21). Therefore, we used [^{111}In]pDNA for the quantitative study of the intrahepatic distribution of pDNA (Fig. 2), expecting a stable retention of the intracellular radioactivity during the isolation steps. Indeed, the intracellular ^{32}P-radioactivity was substantially reduced after the isolation steps (data not shown). However, the amount of degradation product of pDNA could not be determined with [^{111}In]pDNA because [^{111}In]pDNA is unsuitable for the TCA precipitation method. Therefore, [^{32}P]p-DNA was used for the *in vitro* experiments involving cellular association and degradation of pDNA (Figs. 4–6) in order to estimate the amount of extracellular or intracellular degradation products by the TCA precipitation method.

We first examined the *in vivo* disposition of pDNA in rats and showed that pDNA was rapidly eliminated from the circulation and taken up by the liver after intravenous injection (Fig. 1), similarly to our previous findings using mice (3,6). A quantitative analysis of the intrahepatic distribution of [^{111}In]pDNA revealed that the amount of radioactivity taken up by LSECs and Kupffer cells, being much higher than those recovered in hepatocytes, was similar in cell number basis, thereby indicating a large contribution of LSECs to the overall hepatic uptake of pDNA following intravenous injection (Fig. 2), as the liver contains 2.5-fold more LSECs than Kupffer cells (15,16). This supports the fact that inhibition of Kupffer cells by gadolinium chloride did not significantly reduce the total hepatic uptake of [^{32}P]pDNA in mice (6). Taken together, the current findings imply a significant role of LSECs in pDNA clearance, in addition to the previous suggestion that the hepatic uptake of intravenously injected pDNA is largely mediated by Kupffer cells (22).

In uptake experiments with primary cultures of LSECs, the degree of the apparent cellular association of [^{32}P]pDNA was similar at 37°C and 4°C (Fig. 4A), although internalization of [FL]pDNA was observed at 37°C but not at 4°C (Fig. 3). The amount of degradation products of [^{32}P]pDNA in the cellular compartment increased rapidly at 37°C, while it was a small fraction of the applied radioactivity, suggesting that rapid degradation of pDNA occurred in LSECs. At the same time, a dramatic increase in the degradation products of [^{32}P]pDNA in the culture medium was observed at 37°C, whereas there was no increase in radioactivity at 4°C (Fig. 4C). These results suggested that pDNA was internalized and rapidly degraded by LSECs and that degraded fragments of pDNA were readily released into the culture medium. The degradation of [^{32}P]pDNA observed in the current study seems to occur predominantly in the intracellular compartment following internalization or on the surface of the LSECs, but not in the culture medium, as no significant degradation was observed during incubation of [^{32}P]pDNA with medium alone, which had been exposed to LSECs for 3 h (data not shown).

A specific mechanism is involved in the cellular uptake process of pDNA by LSECs as shown in the competitive experiment. pDNA uptake and binding were significantly inhibited by excess unlabeled pDNA and some polyanions such as poly I, dextran sulfate, or heparin, but not by others such as poly C or dextran (Fig. 5). This inhibition pattern highly resembles that of our previous *in vivo* study using mice (6), supporting the hypothesis that LSECs make a major contribution to the hepatic uptake of intravenously injected pDNA. Poly I but not poly C is known to form a base quartet-stabilized four strand helix (quadruplex) (23). This hyperstructure would be highly polyanionic. Similar speculation could be applied to polysaccharides such as dextran sulfate and heparin, because they are also highly sulfated. LSECs express various types of receptors essential for endocytosis, such as mannose receptors (24), Fcγ receptors (25), and various classes of scavenger receptors such as SRA, CD36 (class B), LOX-1 (class E), and SREC (class F) (26). Although we could not conclude at this moment which uptake pathway or receptors are involved in pDNA recognition, the results of competitive inhibition experiments have led to the hypothesis that putative receptor(s) on LSECs might recognize pDNA based on its highly polyanionic nature. We have demonstrated that pDNA is taken up by cultured macrophages (27), dendritic cells (28), and brain microvessel endothelial cells (29) via a specific mechanism resembling scavenger receptors. In addition, class A scavenger receptors (SRA) that recognize a wide variety of anionic macromolecules are unlikely to be responsible for pDNA uptake as shown by *in vivo* and *in vitro* experiments using SRA-knockout mice and peritoneal macrophages from these animals (5). Because the inhibition pattern of pDNA uptake is very similar in those different cell types, the cellular uptake mechanisms might be highly conserved among cells including LSECs. It might not be excluded for *in vivo* situation that the serum cationic proteins or complement proteins interact with circulating pDNA and this could facilitate the recognition and uptake of pDNA by LSECs. However, in our previous study using rat liver perfusion system, pDNA was taken up by the liver nonparenchymal cells in the absence of serum via a similar mechanisms involved *in vivo* following intravenous injection. Because serum-free HBSS was used in the current *in vitro* study to avoid serum nuclease-mediated pDNA degradation before recognition by LSECs, the observed pDNA uptake by LSECs was not dependent on these serum proteins. Though further studies are required to elucidate the mechanism involved in pDNA uptake by LSECs, the high density of negative charges arising from the phosphate groups of pDNA may be an important factor.

It is also suggested that pDNA taken up by LSECs is susceptible to degradation. It is likely that the degradation occurs enzymatically predominantly inside the cells probably by DNase II in lysosomes (30) following internalization via

endocytosis, although degradation on the surface of the cells by membrane-bound DNase is not excluded (22). In fact, the degradation was strongly inhibited by cellular uptake inhibitors (Fig. 6), suggesting that the uptake of pDNA by LSECs via the specific mechanism seems to be indispensable for pDNA degradation. This implies that the specific uptake is associated with the pDNA degradation in the LSECs. Unexpectedly, the degradation of pDNA appeared to be inhibited by poly C. Although poly C showed no inhibitory effect on pDNA uptake and binding (Fig. 5), it might affect pDNA degradation in a nonspecific manner.

In conclusion, the current study has shown that LSECs, as well as Kupffer cells, play a key role in the clearance of circulating pDNA following intravenous injection, providing a larger contribution to the overall hepatic uptake of pDNA. We have demonstrated that naked pDNA is efficiently taken up via a specific mechanism and rapidly degraded by LSECs, the degradation products being released into the extracellular space. These findings provide useful information for pDNA delivery in gene therapy and DNA vaccination procedures.

ACKNOWLEDGMENTS

This work was supported, in part, by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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