The Fate of Plasmid DNA After Intravenous Injection in Mice: Involvement of Scavenger Receptors in Its Hepatic Uptake

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Purpose. We examined the stability and disposition characteristics of a naked plasmid DNA pCAT as a model gene after intravenous injection in mice to construct the strategy of *in vivo* gene delivery systems. ***Methods.*** After the injection of pCAT to the mice, stability, tissue distribution, hepatic cellular localization, and effect of some polyanions on the hepatic uptake were studied. ***Results.*** The *in vitro* study demonstrated that the pCAT was rapidly degraded in mouse whole blood with a half-life of approximately 10 min at a concentration of 100 μg/ml. After intravenous injection, pCAT was degraded at a significantly faster rate than that observed in the whole blood, suggesting that pCAT *in vivo* was also degraded in other compartments. Following intravenous injection of [^{32}P] pCAT, radioactivity was rapidly eliminated from the plasma due to extensive uptake by the liver. Hepatic accumulation occurred preferentially in the non-parenchymal cells. The hepatic uptake of radioactivity derived from [^{32}P] pCAT was inhibited by preceding administration of polyanions such as polyinosinic acid, dextran sulfate, maleylated and succinylated bovine serum albumin but not by polycytidylic acid. These findings indicate that pCAT is taken up by the liver via scavenger receptors on the non-parenchymal cells. Pharmacokinetic analysis revealed that the apparent hepatic uptake clearance was fairly close to the liver plasma flow. ***Conclusions.*** These findings provide useful information for the development of delivery systems for *in vivo* gene therapy.

INTRODUCTION

The development of DNA technology has raised wide interest in the treatment of diseases by gene therapy (1). In particular, major efforts have been made to deliver genes efficiently to potential target cells *in vivo*. A wide variety of approaches have been proposed; e.g., the use of retroviruses (2), adenoviruses (3) and plasmid vectors in combination with specific macromolecular ligands (4–7) and liposomes (8–10). The safety of virus vectors has not been fully established because of its random integration to chromosomal DNA. Therefore, it is worth while to develop *in vivo* gene delivery systems for plasmid DNA using non-viral carriers. Wu et al. (4,5) developed asialoglycoprotein-polylysine-DNA conjugates and succeeded in the hepatic expression of the exogenous gene after i.v. injection. Zhu et al. (10), employing cationic liposomes to deliver a model plasmid, obtained variable gene expression in various tissues *in vivo*. In spite of several reports on successful *in vivo* gene expression, there is scarce information on the disposition of injected plasmid DNA itself in the body. Yet it is obviously necessary to understand the *in vivo* fate of plasmid DNA per se in order to develop efficient and safe gene delivery systems.

In a series of investigations, we have systematically studied the pharmacokinetic properties of various macromolecules in relation to their physicochemical characteristics, such as molecular weight and electric charge. Based on these findings, we have developed different kinds of macromolecular carrier systems for small drugs (11), proteins (12–15), and oligonucleotides (16) and have demonstrated their usefulness for therapeutic use (13,14).

In the present study, we examined the stability and disposition characteristics of a naked plasmid DNA after i.v. injection in mice as a first step towards a strategy to control the disposition characteristics of plasmid DNA. Here we report that the liver plays a critical role in the elimination of injected plasmid DNA from plasma and that its hepatic uptake is mediated by scavenger receptors on non-parenchymal cells.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA; crystallized, M.W. 67 kDa) was purchased from ICN Biomedical, U.S.A. Dextran sulfate (DS; M.W. 150 kDa) was from Nacalai Tesque, Kyoto, Japan. Polyinosinic acid (Poly [I]) and polycytidylic acid (poly [C]) were from Sigma Chemical, Co., St. Louis, MO, U.S.A. [α-^{32}P] dCTP was obtained from New England Nuclear (Boston, MA, U.S.A.). All other chemicals were obtained commercially as reagent-grade products. BSA derivatives, succinylated BSA (Suc-BSA) and maleylated BSA (Mal-BSA), were synthesized and purified as described previously (15).

Preparation of Plasmid DNA

As a model gene, we used a plasmid DNA, pCAT-Control Vector (Promega, Madison, WI), containing the chloramphenicol acetyltransferase (CAT) gene fused to the SV 40 promoter and enhancer element. The plasmid was amplified in the HB 101 strain of *Escherichia coli*, extracted by the alkaline lysis technique and purified by the precipitation with polyethylene glycol (17). The purified plasmid was diluted in sterilized phosphate-buffered saline. The purity was confirmed by 1% of agarose gel electrophoresis. After the electrophoresis, the gel was stained with ethidium bromide and visualized on a transilluminator. The DNA concentration was measured by UV absorption at 260 nm. For the study of disposition characteristics in mice, the plasmid was labeled with [α-^{32}P] dCTP by nick translation (17).

Stability of Plasmid DNA

The stability of pCAT was studied by *in vitro* and *in vivo* experiments. For the *in vitro* stability experiment, an aliquot of 500 μl blood was collected from the vena cava of male ddY mice weighing 25–30 g (the Shizuoka Agricultural

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Co-operative Association for Laboratory Animals, Shizuoka, Japan). pCAT was added to the blood to give a final concentration of 100 μg/ml. After incubation at 37 °C for the indicated times, 100 μl of 100 mM EDTA (pH 8.0) was added to stop the degradation of pCAT by deoxyribonuclease (DNase) in the blood. The blood sample was centrifuged to separate the plasma and the plasmid DNA was extracted from the plasma with an equal volume of phenol/chloroform/isoamyl alcohol (24:24:1). The aqueous phase containing the plasmid DNA was applied to agarose gel (1 %) for electrophoresis as described above. For the *in vivo* stability experiment, plasmid DNA was injected into mouse via the tail vein at a dose of 10 mg/kg of body weight. The calculated initial blood concentration at this dose (250 μg/25 g mouse) is comparable to the concentration of the *in vitro* experiment (100 μg/ml) assuming that the blood volume is approximately 2 ml (8 % of the body weight). At indicated times, 500 μl of blood was collected from the vena cava. The DNA was extracted and electrophoresed as described above.

In Vivo Distribution Experiment

Mice received a 1 mg/kg dose of [^{32}P] pCAT (appropriately diluted with unlabeled pCAT) in sterilized saline by tail vein injection. Blood was collected from the vena cava under the ether anesthesia and centrifuged to obtain the plasma sample. The mice were killed at 0.25, 0.5, 1, 5, 15, 20, or 30 min after injection. At these times the lungs, liver, spleen, kidneys, muscle, brain, gall bladder, and intestine were excised, rinsed with saline, weighed, and subjected to assay. Urine was sampled from the bladder at 1, 15, and 30 min after injection. The radioactivity in the plasma, urine, and tissues was measured using a liquid scintillation counter (LSA-500, Beckman, Tokyo, Japan) after dissolution in Soluene-350 (Packard, Netherlands) followed by the addition of 5 ml of scintillation medium, Clear-sol I (Nacalai Tesque, Tokyo, Japan). Contamination of the plasma in tissue samples was corrected using distribution data of [^{111}In] bovine serum albumin at 10 min after intravenous injection (18).

In Vivo Localization of [^{32}P] pCAT in Different Liver Cell Types

Cellular localization of [^{32}P] pCAT in the liver was determined in different mice at 10 min after injection by fractionating parenchymal (PC) and non-parenchymal cells (NPCs) with differential centrifugation after collagenase perfusion (19). The radioactivity of each cell fraction was determined by liquid scintillation counting as described above.

Inhibition of Hepatic Uptake of [^{32}P] pCAT by Administration of Polyanions

To examine the mechanism of hepatic uptake of pCAT, [^{32}P] pCAT (1 mg/kg) was injected into mice 1 min after administration of poly [C], poly [I], Suc-BSA, Mal-BSA and DS at a dose of 20 mg/kg. At 10 min after injection of [^{32}P] pCAT, plasma and liver were sampled and subjected to radioactivity counting.

Data Analysis

The tissue distribution data of [^{32}P] pCAT were evaluated according to the pharmacokinetic analysis in terms of a clearance and a tissue uptake rate index (15). The total plasma radioactivity concentration, $C_p(t)$, was normalized to percent of dose / ml and analyzed by a biexponential function using the non-linear least-square program MULTI (20):

$$Cp(t) = A \exp(-\alpha t) + B \exp(-\beta t) \quad (1)$$

Total body clearance (CL_{total}) was calculated by dividing the injected dose by the area under the plasma concentration-time curve (AUC) extrapolated to infinite time. Under the assumption of negligible efflux, tissue uptake clearance index per unit weight (CLI_i) was calculated from:

$$CLI_i = C_i / AUC_{0-t} \quad (2)$$

where C_i is the concentration of radioactivity in each organ at time t. Then the apparent hepatic uptake clearance (CL_{liver}) was expressed as follows:

$$CL_{liver} = CLI_{liver} \times W \quad (3)$$

where W (g) is the total wet weight of the liver. Urinary excretion clearance (CL_{urine}) was calculated according to Eq. 2 using the collective amount excreted in urine. In the present study, the organ uptake clearance and the tissue uptake clearance index were calculated using the values up to 1 min after injection in order to minimize the effect of degradation of [^{32}P] pCAT.

RESULTS

In Vitro and *In Vivo* Stability of pCAT

Fig. 1 (A) shows the degradation of pCAT in mouse whole blood at 37 °C at a concentration of 100 μg/ml. The supercoiled type of pCAT was completely changed to the open-circular and linear types within 5 min and then degraded to lower molecular weight products. Densitometric analysis on the fluorescence intensity revealed that the degradation half-life of pCAT was approximately 10 min (data not shown). Fig. 1 (B) shows the fate of pCAT following i.v. injection at a dose of 10 mg/kg, which gives an initial blood concentration corresponding to the value in the *in vitro* stability experiment (100 μg/ml). After i.v. injection, the nature of the degradation products of pCAT in the plasma was similar to that observed in *in vitro*. However the overall degradation rate of pCAT was much more rapid. The open-circular type of pCAT was not already seen at 5 min and moreover even its linear type was not observed at 15 min. These findings suggest that pCAT undergoes degradation in the plasma and other compartments *in vivo*.

Tissue Distribution of [^{32}P] pCAT After Intravenous Injection

Fig. 2 demonstrates the time course of the radioactivity concentration in the plasma, kidneys, spleen, liver, and lungs of after i.v. injection [^{32}P] pCAT into mice. The radioactivity was cleared rapidly from the plasma, and a significant accumulation of radioactivity was observed in the liver. At earlier time points, the concentration in the lungs was relatively high but the variations in these values were large. No significant radioactivity was observed in the gallbladder,

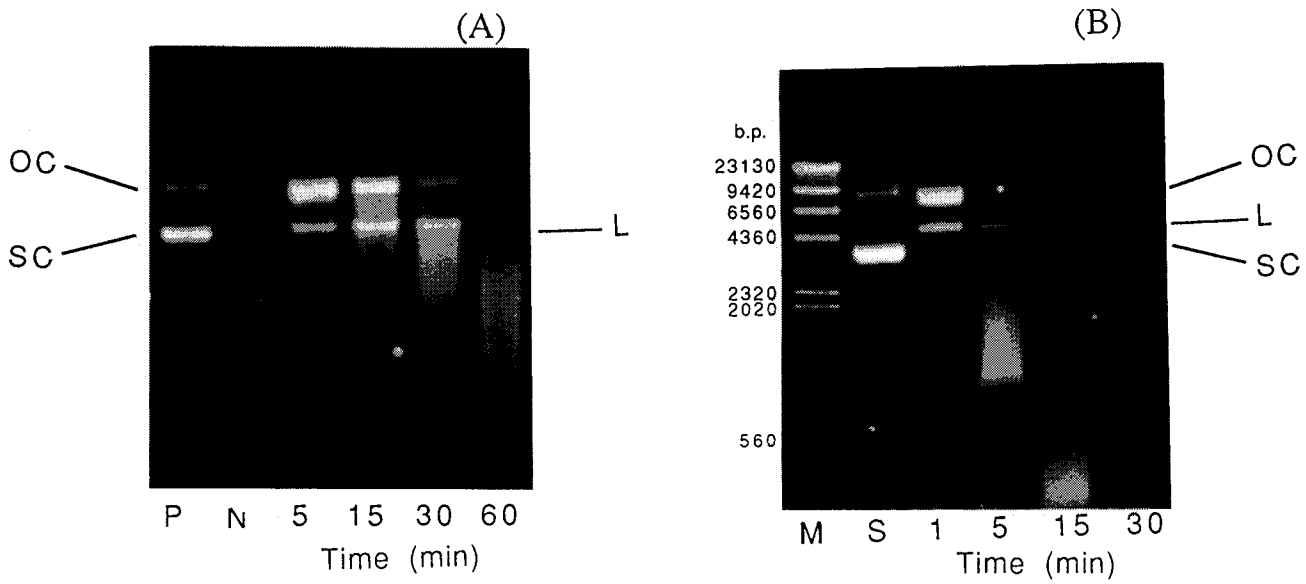

Fig. 1. pCAT stability in mouse whole blood (*in vitro:* A) and after i.v. injection in mice (*in vivo:* B). OC: open circular, L: linear, SC: supercoiled, P: positive control, N: negative control, M: marker(λ/Hind III digest), S: standard. Positive control is the situation where pCAT was put into the EDTA solution before addition of blood. The negative control demonstrates that there is no endogenous DNA in the blood.

intestine, muscle, or brain (data not shown). Fig. 3 shows the amounts of radioactivity in these tissues, together with that excreted in the urine. The extent of hepatic accumulation reached a maximum value of about 70 % of the dose at 5 min and then gradually decreased probably due to returning of degradation products to the plasma pool. A small amount of radioactivity was observed in the spleen and lungs. Both the renal accumulation and urinary excretion of radioactivity increased with time, suggesting that degradation products of [^{32}P] pCAT were excreted via the kidney.

Pharmacokinetic Analysis

Using the plasma concentration data up to 1 min when the influence of degradation was negligible, pharmacokinetic analysis of the plasma disappearance curve was performed. Table I summarizes the AUC, total body-, hepatic- and urinary clearances, as well as the tissue uptake rate index for the organs studied after administration of [^{32}P] pCAT. The

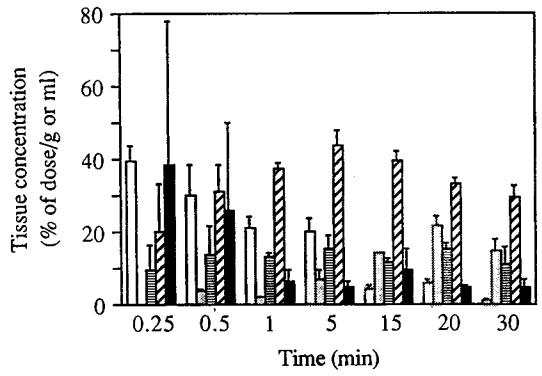

Fig. 2. Concentration of radioactivity in tissues after i.v. injection of [^{32}P] pCAT at a dose of 1 mg/kg in mice. The results are expressed as mean values ± SD of three mice. Key: plasma (□), kidney (▨), spleen (▤), liver (▨), and lung (■).

data of 3′-methoxyethylamine, 5′-biotin decathymidylate (3′M, 5′B-T10) as a model oligonucleotide and Suc-BSA which have already been examined in our laboratory are also shown for comparison (15, 16). This pharmacokinetic analysis revealed a small AUC and a large total-body clearance value of [^{32}P] pCAT in mice that was fairly close to the value of uptake clearance by the liver. The uptake clearance index of liver was also the largest among the tissues examined.

Hepatic Cellular Localization

Fig. 4 shows the distribution of radioactivity in PC and NPC at 10 min after i.v. injection of [^{32}P] pCAT at a dose of 1 mg/kg, together with previously collected data for Gal-BSA, Man-BSA, and Suc-BSA that have been shown to be selectively taken up by these liver cells (15, 21). Like Man-BSA and Suc-BSA, the radioactivity derived from [^{32}P] pCAT was preferentially recovered in the NPC.

Inhibition of Hepatic Uptake of [^{32}P] pCAT by Polyanions

Fig. 5 shows the effect of prior administration of other polyanions (20 mg/kg) on the plasma concentration and liver accumulation of radioactivity after injection of [^{32}P] pCAT (1 mg/kg). [^{32}P] pCAT was administered in the presence of other polyanions, such as poly [I], Suc-BSA, Mal-BSA, and DS, caused a significant increase in plasma concentration and a substantial decrease in hepatic accumulation. Dextran sulfate was the most effective among the polyanions. However, poly [C] was without a significant effect. These findings indicate that the hepatic uptake of [^{32}P] pCAT and probably also of its metabolites is mediated by the scavenger receptors on the NPC.

DISCUSSION

The present study was undertaken with the final aim to

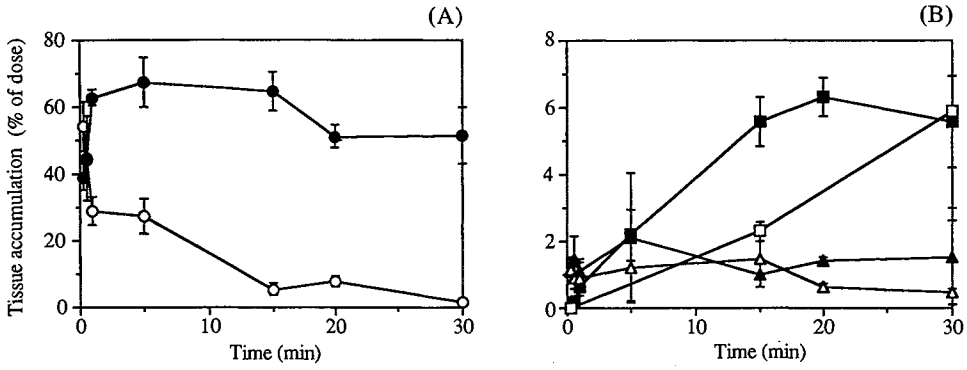

Fig. 3. Tissue accumulation of radioactivity after i.v. injection of [^{32}P] pCAT at a dose of 1 mg/kg in mice. The results are expressed as mean ± SD of three mice. (A) plasma and liver, (B) kidney, spleen, lung, and urine. Key: plasma (○), liver (●), kidney (■), spleen (▲), lung (△), and urine (□).

design a rational delivery system for *in vivo* gene therapy. In this study we demonstrated that uncomplexed pCAT undergoes a rapid degradation *in vitro* and *in vivo*. After i.v. injection in mice, pCAT as well as its degradation products are rapidly taken up by non-parenchymal cells of the liver. In addition, the present findings indicated that the hepatic uptake process involves a scavenger receptor-mediated mechanism. This is the first report, to our knowledge, that demonstrates that plasmid DNA is taken up by the liver nonparenchymal cells via scavenger receptors *in vivo*.

The fate of injected DNA was studied earlier in relation to the potential transforming ability of exogenous DNA (22). In addition to study the pathophysiological mechanism of systemic lupus erythematosus, an autoimmune disease, the fate of exogenous DNA after i.v. injection was examined in mice (23–37). These studies showed that DNA is rapidly removed from the circulation and that the liver plays an important role in this clearance of DNA. The present findings are consistent with these findings.

The *in vivo* experiments show that the degradation rate of pCAT in the circulation of mice was significantly faster than that observed during incubation with the whole blood. This suggests that pCAT is degraded not only by plasma deoxyribonuclease (DNase) (22) but also by enzymes in other body compartments such as the liver. Although the amount of the applied DNA which is extracted from the blood to agarose gel was different for each sample, it is possible to conclude that pCAT is degraded in the blood and other compartments *in vivo*, since there are degradation products at 15 min which is absent in *in vitro* (Fig. 1). Moreover, it is unlikely that the degradation of pCAT observed *in vivo* occurs only after internalization by the liver cells, since the degradation products appeared in the plasma extremely rapidly (Fig. 1). A more likely mechanism in this respect is the involvement of a cell surface DNase (23). To assess this possibility, *in vitro* studies using isolated liver cells are ongoing.

The pharmacokinetic analysis of the initial tissue distribution data of radioactivity, derived from [^{32}P] pCAT up to 1 min after injection, revealed that hepatic uptake clearance is almost identical to the liver plasma flow rate of mice (25 g), being approximately 85,000 μl/hr (28). This suggests that the liver almost completely extracts pCAT. Fig. 6 summarizes the hepatic and urinary clearances of pCAT and other macromolecules which have been previously examined in our laboratory (15, 16). At a later phase following the intravenous injection of [^{32}P] pCAT, the radioactivity accumulated in the liver decreased with time probably due to the release of degradation products into the plasma pool (Fig. 3). In accordance with this idea is the observed increase of radioactivity in the kidney and urine. This indicates that the degradation products are excreted via the kidney. The excretion profile of the degradation products may be similar to that of 3′M, 5′B-T_{10} (Fig. 6). At an early period after administration, the radioactivity concentration in the lung was very high, albeit with large variations (Fig. 2). This suggests some interaction of pCAT with the capillary walls during the first passage after intravenous injection. The involvement of the scavenger receptors in the lung is not likely because pCAT is retained in the lungs for only a very short time. The actual mechanism of lung accumulation remains to be studied. Thus, when the clearance value of the lungs is calculated, it is possible to ignore the efflux of degradation products.

pCAT, which is a polyanion with a molecular weight of

Table I. Pharmacokinetic Parameters of pCAT, Modified Thymidine 10-mer, and Suc-BSA After i.v. Injection in Mice

Compounds	Dose (mg/kg)	AUC (% of dose hr/ml)	Clearance (μl/hr)			Tissue Uptake Clearance Index (μl/hr/g)			
			Total	Urine	Liver	Kidney	Liver	Spleen	Lung
[^{32}P]pCAT	1.0	0.98	102041	0	80379	2674	47751	16726	7788
[^{3}H]3′M,5′B-T_{10}[a]	1.0	1.33	75000	17015	11102	39749	7292	3680	10328
[^{111}In]Suc-BSA[a]	1.0	18.1	5510	393	3763	442	3350	82	327

[a] Data for 3′M, 5′B-T10 and Suc-BSA are published results (15, 16).

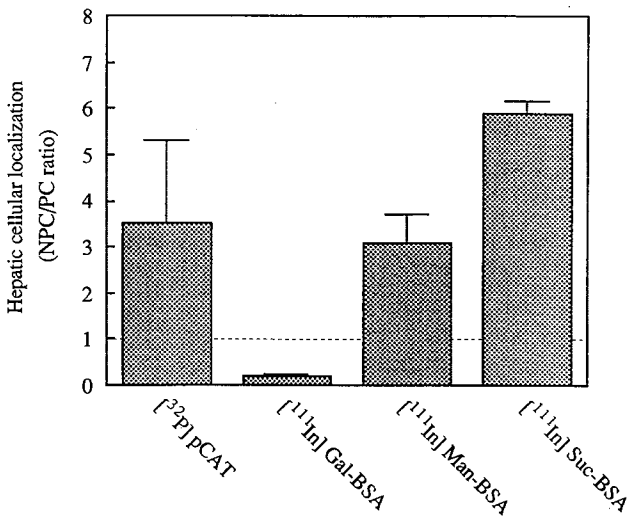

Fig. 4. Hepatic cellular localization of radioactivity at 10 min after i.v. injection of [^{32}P] pCAT at a dose of 1 mg/kg. The amount of accumulation on each cell fraction is calculated to percent of dose per 10^7 cells. Data for Gal-BSA, Man-BSA, and Suc-BSA are published results (15, 21). The results are expressed as mean ± SD of three mice.

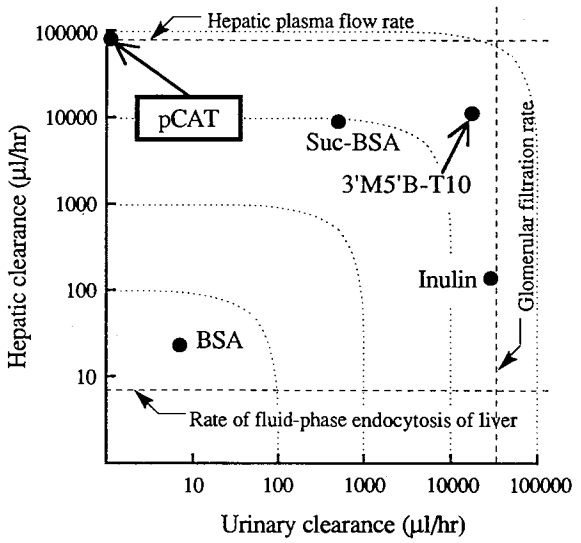

Fig. 6. Hepatic and urinary clearances of macromolecules in mice after i.v. injection. Data except for those on pCAT are published results (15, 16).

about 3,000 kDa, was shown to be taken up preferentially by the NPC of the liver. This suggested that hepatic uptake of pCAT is mediated by scavenger receptors for polyanions on the NPC. Emlen et al. (23) reported that single strand DNA from the calf thymus was bound to hepatic NPC in perfused mouse liver. They concluded that this binding was mediated by an unknown receptor. However, they did not observe a significant inhibition by the anionic protein, ovalbumin. Scavenger receptors recognize a wide range of polyanions including acetylated low density lipoprotein, Mal-BSA, DS, poly [I], and bacterial lipopolysaccharide (29), whereas some other polyanions including poly [C] seem not to be ligands

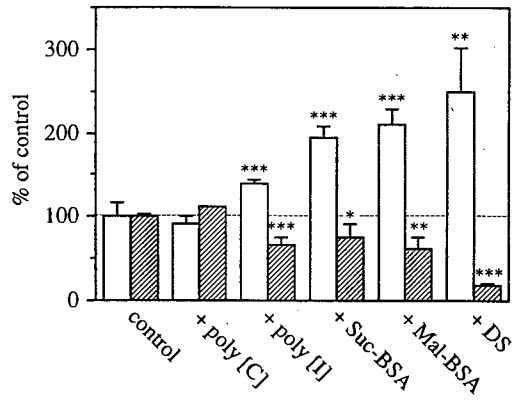

Fig. 5. Competition of hepatic uptake of [^{32}P] pCAT by co-administration with polyanions after i.v. injection into mice. [^{32}P] pCAT (1 mg/kg) was injected at 1 min after administration of unlabeled competitive polyanions (20 mg/kg). Plasma concentration and hepatic accumulation were determined at 10 min after i.v. injection. The values of plasma concentration (% of dose/ml) and hepatic accumulation (% of dose) in the control experiment are 23.9 ± 5.70 and 61.9 ± 0.33, respectively. The results are expressed as mean ± SD of three mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Key: plasma concentration (□), hepatic accumulation (▨).

for the scavenger receptors. It is well known that scavenger receptors exist on the surface of liver NPC, i.e., macrophages (Kupffer cells) and endothelial cells. The findings obtained in competition experiments with these polyanionic compounds (Fig. 5) support the above-mentioned hypothesis.

It remains to be established which type of liver nonparenchymal cells is responsible for the uptake of the plasmid DNA. Kupffer and endothelial cells reportedly have different types of scavenger receptors (30). According to the earlier mentioned finding on single strand DNA (23), Kupffer cells seem to be primarily important for pCAT uptake mediated by scavenger receptors. *In vitro* studies using cultured mouse peritoneal macrophages, that have characteristics similar to Kupffer cells, also support this possibility. These macrophages avidly bound pCAT and the inhibitory effects with the polyanions was almost the same as depicted in Fig. 5 (unpublished data).

The bovine type I and type II macrophage scavenger receptors (31, 32) have been properly characterized. A recent report showed that the binding specificities for polyanionic ligands of these receptors are similar to those of complement component C1q (33). On the other hand, it is known that C1q binds to DNA (34), suggesting that plasmid DNA may bind to these well-known scavenger receptors. Another study has demonstrated that, if polynucleotide interacts with scavenger receptors, a specific conformation is required for a stable binding (35). In the present study, scavenger receptors might recognize some specific sequence(s) in the pCAT molecule.

Thus, the present study reveals some essential aspects on the stability and pharmacokinetic properties of plasmid DNA *in vivo*. These findings showed that improved *in vivo* stability and escape from the recognition by the scavenger receptors on the liver NPC are essential for effective DNA delivery. In order to accomplish these issues, complexation with macromolecular carriers such as polylysine-glycoprotein conjugates (4, 5) or cationic liposomes (10), should

be useful. However, even if plasmid DNA are complexed with these carriers, a part of the complexes may be cleared by the liver NPC when a portion of DNA is recognized by the scavenger receptors. One possible strategy for optimal gene delivery is to utilize chemical modification with polyethylene glycol, which prevents a variety of biological interactions of macromolecules and nanoparticles (36). Currently, further studies are ongoing in our laboratory on these carriers with improved stability for an optimal control of the body disposition of plasmid DNA.

REFERENCES

1. R. C. Mulligan. The basic science of gene therapy. *Science* 260:926–932 (1993)
2. S. J. Szilvassy, C. C. Fraser, C. J. Eaves, P. M. Lansdorp, A. C. Eaves, and R. K. Humphries. Retrovirus-mediated gene transfer to purified hemopoietic stem cells with long-term lympho-myelopoietic repopulating ability. *Proc. Natl. Acad. Sci. USA.* 86:8798–8802 (1989)
3. M. A. Rosenfeld, K. Yoshimura, B. C. Trapnell, K. Yoneyama, E. R. Rosenthal, W. Dalemans, M. Fukayama, J. Bargon, A. Stier, J-P.Lecocq, and R. G. Crystal. *In vivo* transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell* 68:143–155 (1992)
4. G. Y. Wu and C. H. Wu. Receptor-mediated gene delivery and expression *in vivo*. *J. Biol. Chem.* 263:14621–14624 (1988)
5. C. H. Wu, J. M. Wilson, and G. Y. Wu. Targeting genes: Delivery and persistent expression of a foreign gene driven by mammalian regulatory elements *in vivo*. *J. Biol. Chem.* 264:16985–16987 (1989)
6. J. C. Perales, T. Ferkol, H. Beegen, O. D. Ratnoff, and R. W. Hanson. Gene transfer *in vivo:* Sustained expression and regulation of genes introduced into the liver by receptor-targeted uptake. *Proc. Natl. Acad. Sci. USA.* 91:4086–4090 (1994)
7. D. T. Curiel, S. Agrawal, E. Wagner, and M. Cotten. Adenovirus enhancement of transferrin-polylysine-mediated gene delivery. *Proc. Natl. Acad. Sci. USA.* 88:8850–8854 (1991)
8. P. L. Felgner and G. M. Ringold. Cationic liposome-mediated transfection. *Nature* 337:387–388 (1989)
9. J. G. Smith, R. L. Walzem, and J. B. German. Liposomes as agents of DNA transfer. *Biochim. Biophys. Acta.* 1154:327–340 (1993)
10. N. Zhu, D. Liggitt, Y. Liu, and R. Debs. Systemic gene expression after intravenous DNA delivery into adult mice. *Science* 261:209–211 (1993)
11. H. Imoto, Y. Sakamura, K. Ohkouchi, R. Atsumi, Y. Takakura, H. Sezaki, and M. Hashida. Disposition Characteristics of macromolecules in the perfused tissue-isolated tumor preparation. *Cancer Res.* 52:4396–4401 (1992)
12. T. Fujita, M. Nishikawa, C. Tamaki, Y. Takakura, M. Hashida, and H. Sezaki. Targeted delivery of human recombinant superoxide dismutase by chemical modification with mono- and polysaccharide derivatives. *J. Pharmacol. Exp. Ther.* 263:971–978 (1992)
13. T. Fujita, H. Furitsu, M. Nishikawa, Y. Takakura, H. Sezaki, and M. Hashida. Therapeutic effects of superoxide dismutase derivatives modified with mono- and polysaccharide on hepatic injury induced by ischemia/reperfusion. *Biochem. Biophys. Res. Commun.* 189:191–196 (1992)
14. Y. Takakura, S. Masuda, H. Tokuda, M. Nishikawa, and M. Hashida. Targeted delivery of superoxide dismutase to macrophages via mannose receptor-mediated mechanism. *Biochem. Pharmacol.* 47:853–858 (1994)
15. Y. Takakura, T. Fujita, H. Furitsu, M. Nishikawa, H. Sezaki, and M. Hashida. Pharmacokinetics of succinylated proteins and dextran sulfate in mice: Implications for hepatic targeting of protein drugs by direct succinylation via scavenger receptors. *Int. J. Pharm.* 105:19–29 (1994)
16. T. Miyao, Y. Takakura, and M. Hashida. Stability and *in vivo* disposition characteristics of oligonucleotides and oligonucleotide conjugated with macromolecule. Proc. of the 20th Symp. on Controlled Release of Bioactive Materials, Washington, D.C., USA. 492–493 (1993)
17. J. Sambrook, E. F. Fritsch, and T. Maniatis. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed. (1989)
18. Y. Takakura, T. Fujita, M. Hashida, and H. Sezaki. Disposition characteristics of macromolecules in tumor-bearing mice. *Pharm. Res.* 7:339–345 (1990)
19. S. Nakane, S. Matsumoto, Y. Takakura, M. Hashida, and H. Sezaki. The accumulation mechanism of cationic mitomycin C-dextran conjugates in the liver: *In-vivo* cellular localization and *in-vitro* interaction with hepatocytes. *J. Pharm. Pharmacol.* 40:1–6 (1988)
20. K. Yamaoka, Y. Tanigawara, H. Tanaka, and Y. Uno. A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobio-Dyn.* 4:879–885 (1981)
21. M. Nishikawa, Y. Ohtsubo, J. Ohno, T. Fujita, Y. Koyama, F. Yamashita, M. Hashida, and H. Sezaki. Pharmacokinetics of receptor-mediated hepatic uptake of glycosylated albumin in mice. *Int. J. Pharm.* 85:75–85 (1993)
22. Ch. Gosse, J. B. Le Pecq, P. Defrance, and C. Paoletti. Initial degradation of deoxyribonucleic acid after injection in mammals. *Cancer Res.* 25:877–883 (1965)
23. W. Emlen, A. Rifai, D. Magilavy, and M. Mannik. Hepatic binding of DNA is mediated by a receptor on nonparenchymal cells. *Am. J. Pathol.* 133:54–60 (1988)
24. T. Tsumita and M. Iwanaga. Fate of injected deoxyribonucleic acid in mice. *Nature* 198, 1088–1089 (1963)
25. W. Emlen and M. Mannik. Kinetics and mechanisms for removal of circulating single-stranded DNA in mice. *J. Exp. Med.* 147:684–699 (1978)
26. W. Emlen and M. Mannik. Effect of DNA size and strandedness on the *in vivo* clearance and organ localization of DNA. *Clin. Exp. Immunol.* 56:185–192 (1984).
27. F. G. Cosio, L. A. Hebert, D. J. Birmingham, B. L. Dorval, A. P. Bakaletz, G. A. Kujala, J. C. Edberg, and R. P. Taylor. Clearance of human antibody/DNA immune complexes and free DNA from the circulation of the nonhuman primate. *Clin. Immunol. Immunopathol.* 42:1–9 (1987)
28. "Handbook of Physiology," W. F. Hamilton and P. Dow. Eds., American Physiological Society, Washington, D.C., sect. 2. (1962)
29. M. Krieger, S. Acton, J. Ashkenas, A. Pearson, M. Penman, and D. Resnick. Molecular flypaper, host defense, and atherosclerosis: Structure, binding properties and functions of macrophage scavenger receptors. *J. Biol. Chem.* 268:4569–4572 (1993)
30. Y. B. De Rijke and J. C. Van Berkel. Rat liver Kupffer and endothelial cells express different binding proteins for modified low density lipoproteins. *J. Biol. Chem.* 269:824–827 (1994)
31. T. Kodama, M. Freeman, L. Rohrer, J. Zabrecky, P. Matsudaira, and M. Krieger. Type I macrophage scavenger receptor contains α-helical and collagen-like coiled coils. *Nature* 343, 531–535 (1990)
32. L. Rohrer, M. Freeman, T. Kodama, M. Penman, and M. Krieger. Coiled-coil fibrous domains mediate ligand binding by macrophage scavenger receptor type II. *Nature* 343:570–572 (1990)
33. S. Acton, D. Resnick, M. Freeman, Y. Ekkel, J. Ashkenas, and M. Krieger. The collagenous domains of macrophage scavenger receptors and complement component Clq mediate their similar, but not identical, binding specificities for polyanionic ligands. *J. Biol. Chem.* 268:3530–3537 (1993)
34. M. R. Van Schravendijk and R. A. Dwek. Interaction of C1q with DNA. *Mol. Immunol.* 19:1179–1187 (1982).
35. A. M. Pearson, A. Rich, and M. Krieger. Polynucleotide binding to macrophage scavenger receptors depends on the formation of base-quartet-stabilized four-stranded helices. *J. Biol. Chem.* 268:3546–3554 (1993)
36. J. M. Harris, ed. Poly (Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications. New York: Plenum Press (1992)