Pharmacokinetics of Plasmid DNA in the Rat

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Purpose. The pharmacokinetics of plasmid DNA after IV bolus administration in the rat by following supercoiled (SC), open circular (OC), and linear (L) pDNA forms of the plasmid.

Methods. SC, OC, and L pDNA were injected at 2500, 500, 333, and 250 μg doses. The concentrations in the bloodstream of OC and L pDNA were monitored.

Results. SC pDNA was detectable in the bloodstream only after a 2500 μg dose, and had a clearance of 390(±50) ml/min and V_d of 81(±8) ml. The pharmacokinetics of OC pDNA exhibited non-linear characteristics with clearance ranging from 8.3(±0.8) to 1.3(±0.2) ml/min and a V_d of 39(±19) ml. L pDNA was cleared at 7.6(±2.3) ml/min and had a V_d of 37(±17) ml. AUC analysis revealed that 60(±10) % of the SC was converted to the OC form, and nearly complete conversion of the OC pDNA to L pDNA. Clearance of SC pDNA was decreased after liposome complexation to 87(±30) ml/min. However the clearance of OC and L pDNA was increased relative to naked pDNA at an equivalent dose to 37(±9) ml/min and 95(±37) ml/min respectively.

Conclusions. SC pDNA is rapidly metabolized and cleared from the circulation. OC pDNA displays non-linear pharmacokinetics. Linear pDNA exhibits first order kinetics. Liposome complexation protects the SC topoform, but the complexes are more rapidly cleared than the naked pDNA.

KEY WORDS: pharmacokinetics; plasmid DNA; liposomes; supercoiled.

INTRODUCTION

Naked pDNA is being used successfully in gene delivery by local (1–4) and intravenous injection (5–9). The success of these studies indicates a thorough understanding of the pharmacokinetics of naked pDNA is an important area to be considered.

Plasmid DNA pharmacokinetics have been investigated using radiolabeled linear [^{33}P] pDNA (10). These investigations have led to the conclusion that the half-life of the radiolabel is 7 to 12 min after IV bolus administration of naked pDNA in mice. However, this analysis offers information only on the Linear (L) form of the pDNA and no information upon the other functional forms of the plasmid; supercoiled (SC) or open circular (OC), nor does it discriminate the free label. Other studies have qualitatively revealed that the SC topoform of pDNA is not detectable as early as one minute after intravenous administration in mice (11,12). The OC form of the plasmid has a half-life estimated in these studies to be in the range of 10 to 20 minutes (11).

The purpose of this investigation was to evaluate the pharmacokinetics of naked pDNA in a topoform specific manner. Furthermore, we sought to compare the kinetics seen with naked pDNA to those observed after administration of liposome: pDNA complexes.

METHODS

Animals (male Sprague-Dawley rats 300–350 g) were purchased from Charles River Laboratories (Wilmington, MA). Animals were housed in the University of Florida Animal Resources Unit prior to all experiments and were given food and water ad libitum. For all experiments rats were anesthetized by intraperitoneal injection with 0.5 ml of a cocktail containing 13 mg/kg Xylazine, 2.15 mg/kg Acepromazine (The Butler Co., Columbus, OH), and 66 mg/kg Ketamine (Fort Dodge Animal Health, Fort Dodge Iowa).

Phenol: chloroform: isoamyl alcohol (25: 24: 1, v/v/v), chloroform, Tris, boric acid, EDTA, and agarose were purchased from Sigma Chemical Company (St. Louis, MO). Ethidium bromide (electrophoresis grade) was purchased from Fisher Biotech (Fair Lawn, NJ). Competent JM109 bacteria (Promega, Madison, WI) were transformed according to the manufacturers directions with the pGL3 control plasmid (Promega, Madison, WI), pGE150 (a generous gift of Dr. G. Elliot, Marie Curie Research Institute, The Chart, Oxted, Surrey, UK) or pGeneMax plasmid (Gene Therapy Systems, San Francisco, CA). Plasmid DNA was isolated from overnight cultures using alkaline lysis and ultracentrifugation with a CsCl gradient (13). Isolated pDNA was resuspended in phosphate buffered saline. Plasmid was >90% SC by agarose gel analysis.

OC pDNA was produced by incubation of the SC pDNA at 70°C for 16h. This procedure resulted in >90% OC plasmid by agarose gel analysis. UV absorbance at 260 nm and the A260/A280 ratio of the pDNA solution did not change after this treatment. L pDNA was produced by digestion with BamHI restriction enzyme. Plasmid was then isolated from the reaction mixture by extraction with 1 volume of phenol: chloroform: isoamyl alcohol (25: 24: 1), followed by extraction with 1 volume of chloroform. Plasmid was then concentrated by precipitation with 0.3 M Na Acetate, and 1 volume of isopropanol, followed by centrifugation at 13K *g* for 30 min at 4°C, and resuspended in 50 μl of phosphate buffered saline. This method routinely produced 95% L pDNA by agarose gel analysis.

For blood sampling, male Sprague-Dawley rats (300–350g) were anesthetized and the jugular vein cannulated. For injections, the femoral vein was isolated, and pDNA was injected into the femoral vein using a 27-gauge needle. Isolated blood samples (approx. 300 μl) were drawn through the jugular vein cannula and immediately placed in test tubes containing 0.57 ml of 0.34 M EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) on ice at the times indicated in the figures. This concentration of EDTA has previously been shown to inhibit the degradation of pDNA in isolated rat plasma (14).

To isolate pDNA from whole blood samples, 250 μl of blood was liquid/ liquid extracted with 250 μl of phenol: chlo-

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roform: isoamyl alcohol (25: 24: 1, v/v/v), vortexed for 5 s at low speed, and centrifuged at 20,800 *g* for 10 min at room temperature. The aqueous phase was removed and stored at –20°C until analysis.

The three pDNA topoforms were separated by gel electrophoresis as previously reported (14). Accuracy was 96 to 104% for SC pDNA, 98 to 108% for OC, and 96 to 113% for L pDNA. Percent coefficient of variation was ≤ 5%, 19%, and 13% for SC, OC and L forms of the plasmid respectively. Standard curves for all three forms of the plasmid were linear between 10 and 250 ng pDNA (R^2 = 0.9995, 0.9985, and 0.9933 for SC, OC, and L respectively). All reported concentrations were calculated from bands within the range of the standard curves. Lower limit of quantitation was 0.5 ng/μl for all three forms of the plasmid. Lower limit of detection was 0.25 ng/μl for all three forms of the plasmid. Comparisons of the relative fluorescence of SC pDNA versus OC and L pDNA were made by analyzing equivalent amounts as described above and comparing the resulting fluorescence. It was found that on a weight to weight ratio, SC pDNA was only 59% as fluorescent relative to L pDNA by agarose gel analysis. To correct for this difference, SC pDNA amounts were multiplied by 1.7 prior to analysis (15). Percent recovery using the phenol: chloroform: isoamyl alcohol method was found not to be dependent on topoform. Recovery was 90 (±6) % for SC and 86 (±13) % for L pDNA.

All lipids were purchased from Avanti Polar Lipids (Alabaster, AL). Liposomes were prepared by mixing 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and cholesterol in a 1:1 molar ratio as previously described (14). Liposome: pDNA complexes were formed by mixing liposomes with pDNA at a 2:1 lipid: DNA weight ratio and incubating at room temperature for 15 min.

For Southern analysis, an IV bolus of pGL3 was given at a dose of 500 μg via the femoral vein. Thirty seconds after administration, the liver, lung, heart, spleen, kidney, brain, and blood were removed. Isolated tissues were immediately placed in 25 mM Tris-hydrochloride 340 mM EDTA, pH 7.4, and homogenized with a PowerGen 125 (Fisher Scientific, Pittsburgh, PA). Equal aliquots of homogenized tissues were extracted with phenol: chloroform: isoamyl alcohol (25: 24: 1, v/v/v), and centrifuged at 5000 × g. The aqueous phase was removed and DNA was precipitated with 1 volume of isopropanol and 0.3 M Na Acetate. The pellet was resuspended in 500 μl of TE buffer (10 mM Tris-hydrochloride, 1mM EDTA, pH 7.5). Samples were loaded on 0.8% Tris-borate EDTA (TBE) buffer based agarose gels containing 0.001% ethidium bromide. Electrophoresis was carried out at 0.30 V/cm^3 for 12h. The gels were then blotted according to established protocols (16). Southern Blotting was carried out using the North2South Chemiluminescent Nucleic Acid Hybridization and Detection Kit (Pierce, Rockford, IL) with a 5′ biotinylated probe: 5′-ACCTTCTGCGGTTTTTGTATTTCTTTC-CGGGCCGCGGTAAGATAGGCGACCTTCTACCTT-3′ (Life Technologies, Rockville, MD).

Theoretical

SC and L pDNA concentrations were fit to pseudo first-order kinetics. OC pDNA was fit to Michelis-Menten kinetics (17). The degradation of L pDNA is considered to yield fragments of heterogeneous lengths, thus these products were not included in the fitted model. Based on this model the following differential equations were derived to describe the kinetics of pDNA:

$$\frac{dSC}{dt} = -(k_s + k_u) \cdot SC$$

$$\frac{dOC}{dt} = k_s \cdot SC - \frac{V_{\max(OC)} \cdot OC}{(K_{m(OC)} + OC)}$$

$$\frac{dL}{dt} = \frac{V_{\max(OC)} \cdot OC}{(K_{m(OC)} + OC)} - k_L \cdot L$$

Where *SC, OC,* and *L* are the amounts of SC, OC, and L pDNA present at time *t* respectively. The constant k_s represents the rate constant for the degradation of SC pDNA to OC pDNA. The constant k_u represents the uptake rate of SC pDNA into tissues. The parameter $V_{\max(OC)}$ is the maximal rate of elimination of OC pDNA from the circulation. The constant $K_{m(OC)}$ represents the concentration at which the kinetics operate at 1/2 $V_{\max}$ for OC pDNA.

The kinetics of OC pDNA after IV bolus of OC pDNA were fit to a $V_{\max}$ model:

$$\frac{dOC}{dt} = -\frac{V_{\max(OC)} \cdot OC}{K_{m(OC)} + OC}$$

$$\frac{dL}{dt} = \frac{V_{\max(OC)} \cdot OC}{K_{m(OC)} + OC} - k_L \cdot L$$

The kinetics of L pDNA after IV bolus of L pDNA were fit to:

$$\frac{dL}{dt} = -k_L \cdot L$$

Non-linear curve fitting and statistical analysis was carried out using Scientist (version 4.0, Micromath, Salt Lake City, UT). Area under the plasma concentration time curve (AUC) was calculated using trapezoidal rule with extrapolation of the terminal AUC by integration (17). Pharmacokinetic analysis was carried out using standard pharmacokinetic parameters (17). Statistical analysis was performed using SAS (Version 6.12, The SAS Institute, Cary, NC).

RESULTS

Non-Compartmental Analysis

SC pDNA was detectable in the bloodstream only after a 2500 μg dose, no SC pDNA was detectable in the bloodstream at lower doses as early as 30 sec after administration. Because of this, the pharmacokinetic parameters reported for this form of pDNA relied only on data acquired from this dose. SC pDNA remained detectable in the plasma through 1 min after administration. Using the limited available data we approximated that SC pDNA degraded with a half-life of 0.15 (±0.01) min. The degradation of SC pDNA was fit to a one-compartment body model with central elimination. A least squares fit of the data was extrapolated to an initial, t = 0, concentration which was necessary as this area accounted for a major portion of the AUC_∞ (80 ± 3%). Clearance of SC pDNA was estimated to be 390 (±50) ml/min, and volume of distribution was 148 (±26) ml.

To investigate the percent of SC plasmid converted to OC as well as the conversion of OC to L, we compared the AUC obtained after IV bolus administration of each of the forms of plasmid independently at 2500 and 250 μg doses. As is displayed in Figure 1 A and B, OC AUC after administration of SC pDNA was only 64 (±11)% and 59 (±11) % of the AUC after administration of OC pDNA for the 2500 and 250 μg doses respectively. The AUC of L pDNA after administration of OC pDNA was 107 (±24) and 94% (±11) of the AUC of the AUC after administration of L pDNA for the 2500 and 250 μg doses respectively (Figure 1 C and D).

Noncompartmental analysis of all four doses is displayed in Table I for OC and Table II for L pDNA. Clearance of the OC form of the plasmid decreased with increasing dose (Table I). Formation clearance values for the OC form of the plasmid after the administration of SC pDNA ranged from 1.3 (±0.2) to 8.3 (±0.8) ml/min for the 2500, and 250 μg doses respectively. Volume of distribution was 37 (± 19) ml. Corresponding plots of OC pDNA plasma concentrations, normalized for dose, were not superimposable (Figure 3).

Formation clearance of the L form after administration of SC pDNA remained constant at an average of 6.7 (± 0.2) ml/min. The data for the L form after administration of SC pDNA at the 250 μg dose was not included in the noncompartmental analysis as only 3 to 4 time points were above sensitivity limits. Pharmacokinetic parameters for L pDNA after administration of SC pDNA are presented in Table II.

Model-Fitting

We next sought to construct a model to describe the pharmacokinetics of pDNA after IV bolus administration. The model used is outlined in the methods section. SC pDNA was fit to first order kinetics with uptake and degradation to the OC form. Noncompartmental analysis of the OC form of the plasmid suggested nonlinear processes, thus OC pDNA was fit to Michaelis-Menten elimination. Linear pDNA was fit to first order elimination. Plasma concentrations and resulting fitted data for the OC and L pDNA are presented in Figure 2. After administration of SC pDNA, OC pDNA was eliminated with an overall average V_{max} of 1.7 (±0.5) ng/μl/min and a K_m of 7.1 (± 2.1).

Metabolite Analysis

To draw a comparison between the observed pharmacokinetics of the OC form of the plasmid, after injection of SC pDNA, versus after injection of the OC form of the plasmid itself, we injected this metabolite independently at 2500 and 250 μg doses. Unlike the SC form of the plasmid, the OC form of the plasmid was detectable and remained detectable in the bloodstream through 20 min after administration of the 250 μg dose. The elimination of OC pDNA was more appropriately described by a Michaelis-Menten elimination with a statistical improvement of fit observed after utilization of this model over a first-order elimination model (Figure 3 A and B). OC pDNA was eliminated with a V_{max} of 1.0 (±0.3) ng/μl/min and a K_m of 3.9 (±0.9) ng/μl.

The elimination of L pDNA was also investigated after 2500 and 250 μg doses. Resulting plasma concentrations are displayed in Figure 3. The L pDNA also exhibited slower elimination than the SC topoform, remaining detectable in the bloodstream through 7.5 min after administration of a 250

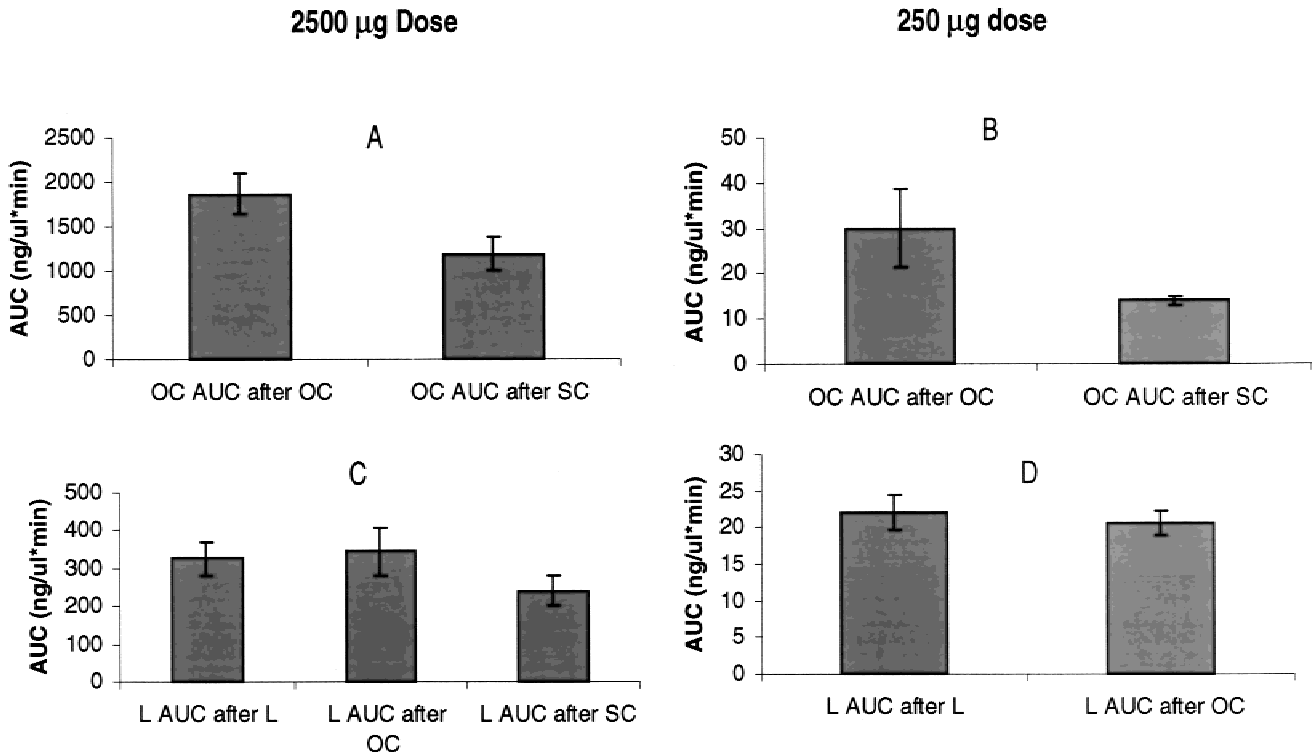

Fig. 1. (A) Area under the curve of OC pDNA after administration of either 2500 μg dose of SC or OC pDNA. (B) Area under the curve of OC pDNA after administration of either 250 μg dose of SC or OC pDNA. (C) Area under the curve of L pDNA after administration of a 250 μg dose of L, OC, or SC pDNA. (D) Area under the curve of L pDNA after administration of a 250 μg dose of L, OC, or SC pDNA. Error bars represent ± 1 standard deviation.

Table I. Non-Compartmental Analysis of OC pDNA after IV Bolus of Increasing Doses of SC pDNA[a]

Parameter	2500 μg Dose	500 μg Dose	333 μg Dose	250 μg Dose
AUC (ng/μl*min)	1200 (±200)	120 (±50)	59 (±3)	18 (±2)
AUC % extrapolated	1 (±0.4)	9 (±4)	10 (±6)	24 (±2)
AUMC (ng/μl*min^2)	20000 (±6000)	1900 (±1200)	400 (±20)	130 (±20)
MRT (min)	16 (±3)	14 (±3)	6.8 (±0.4)	7.2 (±0.3)
Cl/f (ml/min)	2.1 (±0.4)	4.8 (±2.0)	5.7 (±0.3)	14 (±1)
Cl (ml/min)	1.3 (±0.2)	3.0 (±1.2)	3.5 (±0.2)	8.3 (±0.8)
Vd_{ss}/f (ml)	35 (±2)	72 (±32)	39 (±4)	100 (±10)
Vd_{ss} (ml)	20 (±2)	44 (±20)	24 (±2)	60 (±4)
C_{max} (ng/μl)	49 (±4)	13 (±4)	6.5 (±0.3)	2.2 (±0.2)
t_{max} (min)	1	1	0.7 (±0.3)	0.8 (±0.3)

[a] Parameters represent averages (±1 standard deviation) of n = 3 rats/dose.

μg dose. The concentrations of the L form were fit to mono-exponential elimination and had a half-life of 2.1 (± 0.2) min. The fitted elimination rate constant for L pDNA was not significantly different after administration of OC or L pDNA.

Plasmid Sequence Effect

We next explored the effects of plasmid sequence on the observed pharmacokinetics. We investigated this using the pGL3, pGE150, and pGeneMax plasmids. For comparison, the terminal elimination of OC and L were compared after a 500 μg dose of SC pDNA of each of the plasmids. There were no significant differences between pGL3, pGeneMax, or pGE150 in the terminal elimination of each of the plasmids by analysis of variance at the $p<.05$ level.

Tissue Distribution

Thirty seconds after 500 μg dose, the lung liver, kidney, spleen, brain, heart, and blood were removed and were probed for the presence of the plasmid by Southern Blot. As is displayed in Figure 4, the presence of the plasmid was detected in all tissues at approximately equal intensities, with the exception of the brain, which exhibited little signal.

Table II. Non-Compartmental Analysis of L pDNA after IV Bolus of Increasing Doses of SC pDNA[a]

Parameter	2500 μg Dose	500 μg Dose	333 μg Dose
AUC (ng/μl*min)	240 (±40)	52 (±25)	32 (±5)
AUC % extrapolated	12 (±7)	15 (±5)	13 (±7)
AUMC (ng/μl*min^2)	7500 (±2700)	570 (±370)	300 (±20)
MRT (min)	31 (±6)	10 (±2)	9.6 (±1.7)
Cl/f (ml/min)	10.6 (±2.0)	11 (±5)	11 (±1)
Cl (ml/min)	6.5 (±1.2)	6.9 (±2.8)	6.6 (±0.9)
Vd_{ss}/f (ml)	320 (±20)	110 (±30)	100 (±30)
Vd_{ss} (ml)	190 (±80)	64 (±19)	65 (±20)
C_{max} (ng/μl)	5.4 (±0.6)	3.2 (±1.0)	2.4 (±0.5)
t_{max} (min)	22 (±3)	5.3 (±4.0)	6.0 (±3.6)

[a] Parameters represent averages (±1 standard deviation) of n = 3 rats/dose. 250 μg data excluded due to insufficient data.

Lipid Complexation

We next investigated the pharmacokinetics of SC pDNA when complexed to a commonly used cationic lipid *in vivo*. Resulting plasma concentrations of SC, OC and L pDNA are presented in Figure 5. Noncompartmental analysis is presented in Table III. SC pDNA was readily detectable through 5 min after administration of the 500 μg dose. Clearance of SC pDNA was decreased relative to naked pDNA at 87 (±30) ml/min. Vd_{ss} of the SC form of the plasmid also decreased to 79 (±16) ml. However the Cl/f of the OC form of the plasmid increased, relative to administration of naked pDNA at equivalent dose, to 37 (±9) ml/min. Vd_{ss}/f of the OC form of the plasmid increased to 120 (±60). The Cl/f of L pDNA was also increased relative to naked pDNA at 95 (±37) ml/min. Vd_{ss}/f of the L form increased to 330 (±230) ml.

DISCUSSION

DNase I is an enzyme in human plasma present at concentrations averaging 26.1 (±9.2) ng/ml (18). Traditionally the presence of this enzyme has led to the conclusion that pDNA administered intravenously is degraded in a rapid fashion (19,20). This has led to the current view of gene delivery, in which protection from plasma nucleases is a major goal of delivery vehicles. The results of this study reveal that although the half-life of SC and OC pDNA is remarkably short in plasma, degradation alone was not enough to explain the rapid disappearance of pDNA from the circulation.

If we compare the reported concentrations of DNase I in human plasma along with the reported SC pDNA nicking activity of DNase I under optimal conditions (21), and make the assumption that the activity of rat DNase I is similar (22), we can arrive at an approximate activity of 0.1 ng pDNA/μl/min. This is far less than the *in vivo* SC pDNA nicking rate of 9.2 ng pDNA/μl/min observed 30 sec after administration of the 2500 μg dose. One minute after administration the *in vivo* rate was 2.7 ng pDNA/μl/min.

Other investigators have qualitatively commented on this rapid clearance observed after IV bolus administration of SC pDNA (11, 12, 23). Osaka et al (10) utilized a dose of 2.25 μg/g of linearized radiolabeled plasmid and found the half-life to be 6.6 and 11.5 min (n = 2). This corresponds to an approximate dose of 730 μg in a rat. We found the half-life of linear

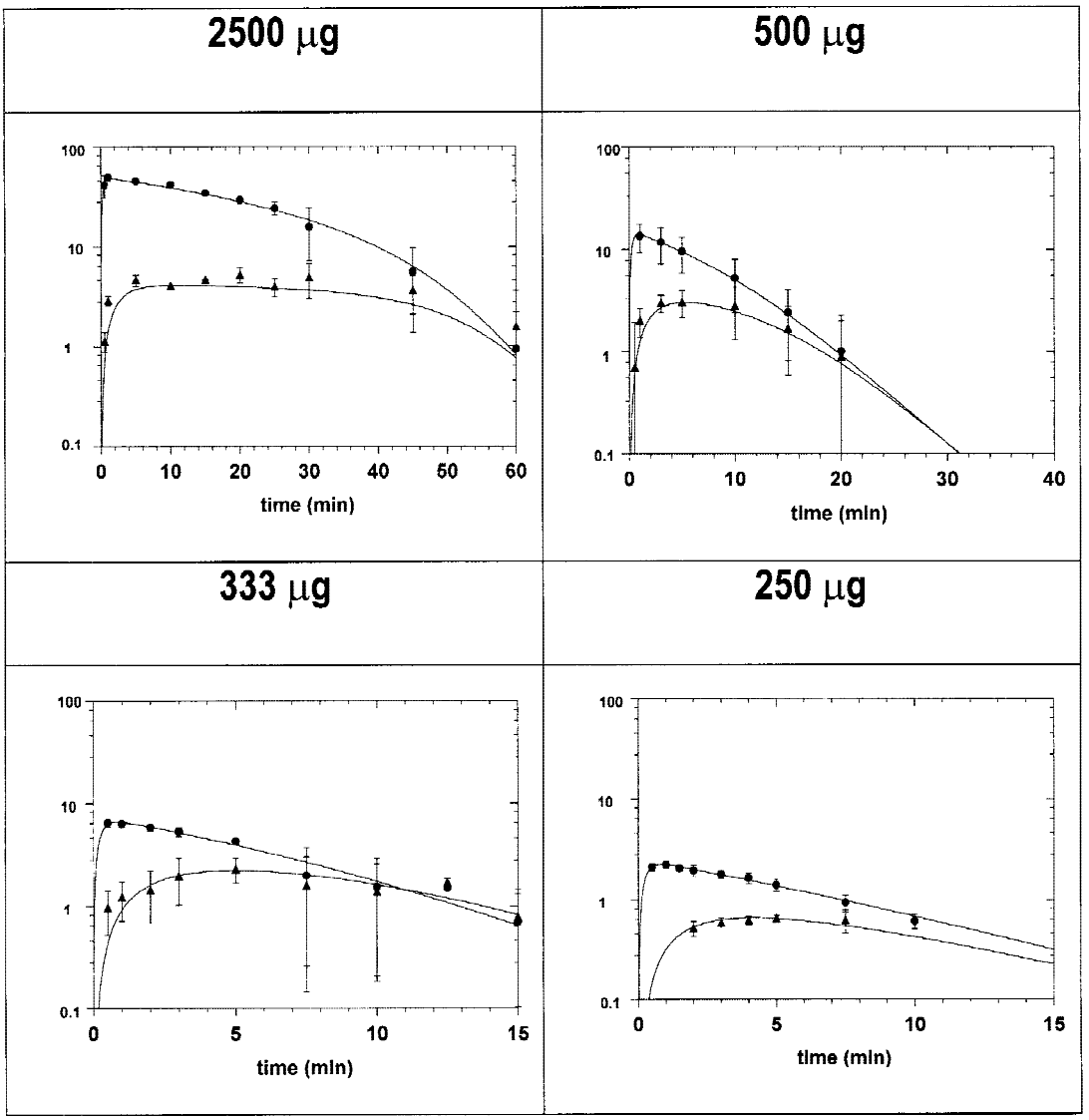

Fig. 2. Fitted and observed values of OC pDNA (●) and L pDNA (♦) after 2500, 500, 333, and 250 μg doses of SC pDNA. Error Bars represent ± 1 standard deviation of n = 3. Data points represent observed data lines represent predicted data.

pDNA to be 2.1 (± 0.2) in our experiments. This difference may be due to species variation and an inability to differentiate the free radiolabel. Thierry et al (11) utilized electrophoresis and estimated the half-life of the OC form of the plasmid to be in the range of 10 to 20 min at a dose of 3.5 μg/g in mice. This corresponds to a dose of approximately 1100 μg in a rat and is in reasonable agreement with the terminal half-lives we observed here between the 500 and 2500 μg doses. Thus, tracing of naked pDNA kinetics using radiolabeling may produce different results than those obtained using electrophoresis.

One possible explanation for the rapid disappearance of the SC pDNA is that it is rapidly being converted to the OC form *in vivo*. However, if this were the case, the AUC of OC pDNA after administration of SC pDNA would be nearly equal to the AUC after administration of OC pDNA. However, the AUC of OC was only 60 (±10) % of the AUC obtained after OC, leaving ~40% of the plasmid to be accounted for by entrapment, association, or conversion to a form undetectable using this method.

It must also be considered that the reason for the longer mean residence time of the OC form of the plasmid may be due to a capacity limited scavenger. It is possible that SC and OC pDNA are cleared by a similar mechanism, but this system is rapidly saturated with the SC form of the plasmid. This would result in the OC pDNA formed being spared from this elimination pathway, and explain the increased residence time in the circulation. If this were the case, it would be expected that the OC form kinetics would more closely resemble the SC kinetics after administration of the OC form itself. However, when the OC form was injected independently, there was no rapid clearance of the plasmid. Both the OC and L pDNA were detectable for as long as 20 min after administration of a 250 μg dose.

The mechanism for the rapid clearance of SC pDNA, relative to the other 2 forms, deserves further investigation.

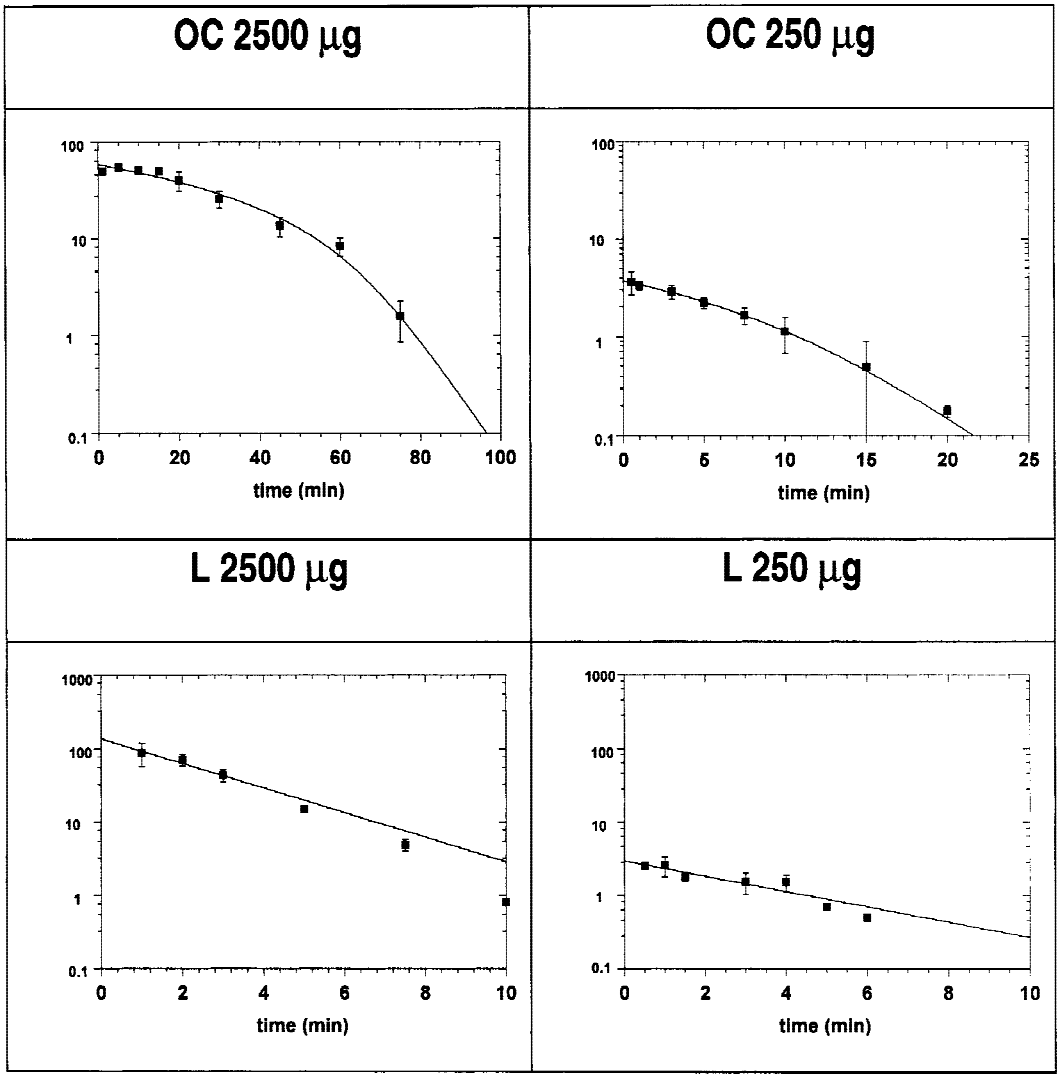

Fig. 3. Average concentrations (ng/μl) of OC and L pDNA in the bloodstream after IV bolus administration of 2500 μg or 250 μg OC or L pDNA. Error bars represent ± 1 standard deviation of n = 3. Data points represent observed data lines represent predicted data.

Previous studies comparing SC pDNA to L pDNA have shown that SC pDNA has stronger acidity than L pDNA (24). This difference is the result of the density and availability of the free phosphate groups. Acidic groups located at the external loops of SC molecules would be available and involved in interactions, while most of the phosphate groups localized within the SC molecule would not interact with components in the bloodstream. OC and L pDNA however likely expose a much higher number of available acidic functional groups (24). These anionic charges would be located all along the pDNA molecules and allow for multiple interactions. This decreased binding affinity of SC pDNA has been displayed in interactions with silica (25) and clay minerals (24). This difference could potentially explain the rapid clearance of SC pDNA relative to OC and L pDNA. Furthermore, this association of OC and L pDNA with plasma components may also offer some protection from plasma nucleases. Protection from nucleases has been displayed after adsorption to proteins and is the basis for DNase I footprinting (26).

Plasmid DNA was detected in the lungs, liver, heart, kidney, and spleen with a low level of detection in the brain. The signal from the brain could be expected to be arising from residual blood as well as pDNA interacting with vascular endothelia, given that pDNA is unlikely to pass through the blood brain barrier. Thus, this level of signal should be taken into account for all tissues. This wide distribution of plasmid could potentially be due to the cells of the reticuloendothelial system (27). Trapping in the micro-vasculature of the plasmid is unlikely, given that the free plasmid has a diameter of approximately 8 to 22 nm (28), while the microvasculature is generally considered to be in the range of approximately 5 μm (27).

The OC pDNA displayed kinetics consistent with saturable elimination. Nonlinear elimination of OC pDNA has previously been suggested using pharmacokinetic analysis of outflow patterns from rat perfused liver studies with radiolabled OC pDNA (29). In this study V_d increased and extraction ratio decreased as perfusion dose was increased from 1.33 to 13.3 μg/liver.

Liposome complexation resulted in a significant change in the pharmacokinetics. If we assume that naked SC pDNA follows first order kinetics, Cl/f of SC pDNA was decreased

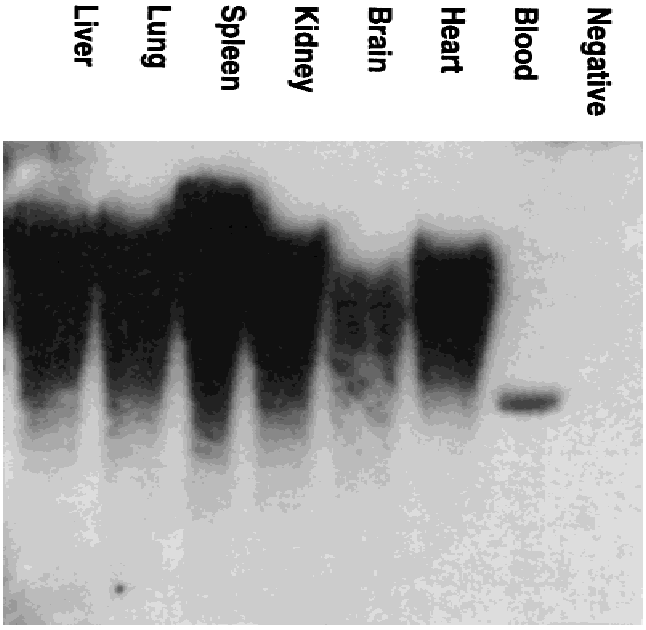

Fig. 4. Southern blot of tissues 30 sec after IV bolus administration.

after liposome complexation suggesting that the SC topoform was protected. The Vd_{ss}/f also decreased suggesting a greater amount of the SC pDNA remained in the circulation. However, Cl/f of OC and L pDNA was increased after administration of liposome: pDNA complexes. This suggests that either there is a change in the fractional formation of OC and L pDNA or that, although the pDNA is spared from degradation, there is a more rapid uptake of the liposome: pDNA complex particle than of the naked OC pDNA. This finding was mirrored by the [^{33}P]pDNA results of Niven et al (30) who found that 36 % of the pDNA dose could be recovered in the bloodstream at 5 min after administration of naked pDNA and only 2.9 % of the dose could be recovered at 5 min after administration of liposome: pDNA complexes. Osaka et al (10) found similar results 2 minutes after administration of liposome: [^{33}P] pDNA complexes with 6.12 % of dose equivalents/g in the blood after administration of liposome:pDNA complexes versus 15.79 % of dose equivalents/g after administration of free [^{33}P] pDNA.

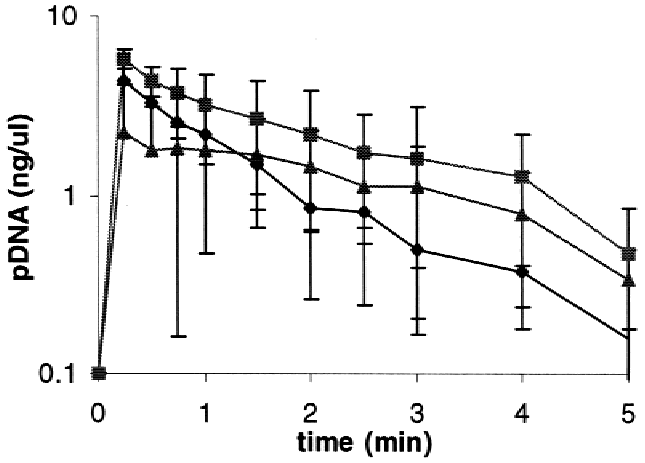

Fig. 5. Pharmacokinetics of pDNA after IV bolus administration of a 500 μg dose of SC pDNA complexed to DOTAP: Cholesterol liposomes. Key: ♦ SC, ■ OC, and ▲ L. Error bars represent ± 1 standard deviation of n = 3.

Table III. Pharmacokinetic Parameters after 500 μg Dose of pDNA Complexed with DOTAP: Cholesterol Liposomes[a]

Parameter	SC	OC	L
AUC (ng/μl*min)	6.3 (±2.3)	14 (±4)	5.7 (±1.9)
AUC (% extrapolated)	12 (±3)	25 (±18)	68 (±26)
AUMC (ng/μl*min^2)	6.8 (±5.3)	40 (±4)	17 (±3)
MRT (min)	0.99 (±0.42)	3.0 (±0.9)	3.3 (±1.0)
Cl/f (ml/min)	87 (±30)	37 (±9)	95 (±37)
Vd_{ss}/f (ml)	79 (±16)	120 (±60)	330 (±230)
C_{max} (ng/μl)	4.4 (±0.2)	5.8 (±0.7)	1.8 (±0.8)
t_{max} (min)	0.25	0.25	0.25

[a] Parameters represent averages (±1 standard deviation) of n = 3 rats.

These results indicate that naked SC pDNA is cleared rapidly from the rat circulation after IV bolus administration at 390 (±50) ml/min, and has a V_d of 148 (±26) ml. The OC form of the plasmid exhibits nonlinear characteristics with clearance ranging from 1.3 (±0.2) to 8.3 (±0.8) ml/min for the 2500 and 250 μg doses respectively. V_d of the OC form was 39 (± 19) ml. The L form of the plasmid is cleared at 7.6 (±2.3) ml/min and has a V_d of 37 (± 17) ml.

AUC analysis revealed that 60 (±10) % of the SC pDNA degraded to the OC form of the plasmid. The conversion of the OC form of the plasmid to the L form of the plasmid appears to be nearly complete as determined by AUC analysis. There is a diffuse tissue distribution of the pDNA 30 seconds after administration with detectable levels in the lung, liver, heart, spleen, and kidneys.

Administration of liposome: pDNA complexes results in a relative maintenance of the SC topoform. Clearance and Vd_{ss} are decreased for SC pDNA after liposome complexation. The OC and L forms of the plasmid exhibit increased Cl/f and Vd_{ss}/f after liposome complexation. These results may suggest a change in the fractional formation of the OC and L pDNA from SC pDNA or that the complexes are overall more rapidly removed from the circulation than naked pDNA while providing some protection of the SC topoform.

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