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Research Article

Liposomal Formulation Eliminates Acute Toxicity and Pump Incompatibility of Parenteral Cyclosporine

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The currently available intravenous dosage form of cyclosporine (CSA), Sandimmune I.V., contains the vehicle, Cremophor EL, which has been implicated in producing anaphylactic reactions in man and animals. This formulation also leaches through silicone tubing, an important component of some automatic drug delivery devices, causing pump dysfunction. In an attempt to develop a less toxic and pump-compatible formulation of CSA, suitable for intrarenal infusion in a canine transplant model, we compared the acute toxicity, pharmacokinetics, and pump compatibility of emulsified (CSA/emulsion) and liposomal (CSA/liposomes) CSA preparations with those of Sandimmune I.V. and CSA dissolved in ethanol vehicle (CSA/alcohol) in healthy, unoperated dogs. Animals receiving Sandimmune I.V. demonstrated marked acute toxicity despite progressive 10-fold dose reduction and >50-fold prolongation of infusion duration. One of two animals receiving CSA/emulsion and both dogs receiving emulsion vehicle alone exhibited a moderately severe reaction, while five of seven dogs receiving CSA/alcohol demonstrated immediate, mild reactions. No discernible adverse reactions occurred in any animal receiving CSA/liposomes. Systemic disposition of CSA/alcohol and CSA/liposomes was similar. In contrast to the liposomal vehicle, the emulsion vehicle produced a marked, early weight gain and substantial decrease in tensile strength of the pump tubing, both of which would adversely affect pump function. These results provide the first description of liposomal CSA toxicology and pharmacokinetics in a large animal model and may lead to the successful development of a less toxic parenteral CSA formulation for systemic and local pump-based administration.

KEY WORDS: liposome; cyclosporine; acute toxicity; pharmacokinetics.

INTRODUCTION

We are reexploring the hypothesis that the administration of immunosuppressive agents directly into a functioning organ transplant will simultaneously prevent rejection and diminish or eliminate systemic side effects. To this end, we have developed a canine renal allograft model utilizing a programmable, implantable pump for intrarenal infusion (1) and have investigated the pharmacokinetic parameters of continuous drug delivery to the autotransplanted kidney (2). Desirable characteristics of an agent to be utilized in our model are (i) potent immunosuppressive activity, (ii) compatibility with pump components, and (iii) high first-pass renal extraction and/or rapid systemic clearance (3). In addition, the agent should not produce nephrotoxicity or acute systemic drug reactions and should be stable at high concentrations for long periods of time.

Cyclosporine (CSA) is a potent immunosuppressant which has now become an integral part of virtually all multiple-drug regimens for preventing rejection in renal, hepatic, cardiac, and pancreatic transplantation. However, the currently available intravenous dosage form of CSA, Sandimmune I.V. (Sandoz Pharmaceuticals Corporation, East Hanover, N.J.), possesses undesirable pharmacokinetic and physicochemical properties and produces both target organ and systemic toxicity. Sandimmune I.V. contains the lipophilic CSA together with the carrier, Cremophor EL, a polyoxyethylated castor oil base in which the drug is dissolved. There are multiple reports of anaphylactic reactions and histamine release after Cremophor EL administration in man (4–6) and animals (7–9), and intravenous administration

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of Sandimmune I.V. has been associated with anaphylactic reactions (10–14) and adult respiratory distress syndrome (15) in transplant patients. Furthermore, Cremophor El administered alone, either systemically or to the isolated kidney, has produced acute reductions in renal blood flow and glomerular filtration rate in rats (16,17). Finally, we have noted deleterious effects of Sandimmune I.V. on pump tubing material, decreasing tensile strength by 10% over a 2-week incubation period. These observations point to the need for development of a new, nontoxic parenteral dosage form of CSA. By changing the drug vehicle, one could substantially reduce local and systemic side effects and enhance pump compatibility, thus making intrarenal CSA administration a more feasible approach.

Several other hydrophobic drugs in Cremophor-EL containing formulations have produced both local and systemic toxicity (4–6,8,9). Preparation of emulsion formulations and encapsulation within liposomes are two techniques which have been used to reduce the toxicity of these drugs. Emulsion preparations of both diazepam and the anesthetic 2,6-diisopropylphenol were better tolerated than the corresponding Cremophor El-containing agents in human and animal studies, respectively (9,18). Liposomal delivery of CSA in rats reduces both renal and systemic toxicity compared to Sandimmune I.V. (19,20) and appears to have equivalent immunosuppressive activity both *in vitro* (19) and *in vivo* (21). Therefore, in an attempt to develop a suitable parenteral formulation of CSA for intrarenal infusion in our canine allograft model, we compared the acute toxicity, pharmacokinetics, and pump compatibility of emulsified (CSA/emulsion) and liposomal (CSA/liposomes) CSA preparations with those of Sandimmune I.V. and CSA dissolved in ethanol vehicle (CSA/alcohol) in healthy, unoperated dogs.

MATERIALS AND METHODS

Cyclosporine Formulations

Sandimmune. Sandimmune I.V. was obtained from our hospital pharmacy in 5-ml vials containing 50 mg/ml CSA and 650 mg/ml Cremophor EL in 32.9% ethanol and diluted in saline to give a final CSA concentration of 10 mg/ml.

CSA/Emulsion. CSA powder (Sandoz Pharmaceuticals Corporation) was dissolved in triacetin and mixed with ethyl oleate. A stock solution of pluronic F68 in distilled water (25 mg/ml) was prepared, and appropriate volumes of this solution were added to the oil phase and vortexed for 1 min. The emulsion was formed by probe sonication for 3 min using an ultrasonic processor (Model W375, Heat Systems, Farmingdale, N.Y.). The final preparation contained 42.5% triacetin, 10% ethyl oleate, 1.14% pluronic F68, and q.s. 100% distilled water, with a CSA concentration of 10 mg/ml. The particles were homogeneously distributed, with a mean diameter of 0.28 μm as measured by a laser particle sizer (Nicomp Model 370, Pacific Scientific, Calif.). This formulation was the most stable at room temperature of several emulsions tested, with no change in particle size over an 8-week period.

CSA/Alcohol. CSA powder was dissolved in 95% ethanol and mixed with an equal volume of normal saline to obtain a final preparation containing 10 mg/ml CSA in 47.5% ethanol.

CSA/Liposomes. Liposomes were prepared using the conventional film method (22) with the drug included in the lipid film. Egg phosphatidylcholine (Avanti Polar Lipids, Inc., Birmingham, Ala.), cholesterol (Sigma Chemicals, St. Louis, Mo.), dicetylphosphate (Sigma Chemicals, St. Louis, Mo.), and CSA powder were mixed in a round-bottom flask at a molar ratio of 1:0.33:0.1:0.07 and dissolved in a mixture of chloroform and methanol. The solvents were removed by rotary evaporation to obtain a dry film. The film was then hydrated using phosphate-buffered saline (PBS) with constant shaking. After the film was homogeneously dispersed, the suspension was bath-sonicated for 5 to 10 min. The final preparation for intravenous injection contained 10 mg/ml CSA and 100 mg/ml egg phosphatidylcholine.

The liposomes were sized using the laser particle sizer and found to be homogeneously distributed, with >90% of the population having a mean diameter between 1.1 and 1.4 μm. To maintain sterility of the preparations, all glassware was sterilized by autoclaving, PBS was passed through a 0.22-μm membrane filter, and the entire procedure was carried out in a laminar flow hood. All preparations were tested in a bacterial growth detector (Bactec Model 300, Johnston Laboratories, Towson, Md.) and found to be sterile for at least 1 week.

Intravenous Pharmacokinetics and Acute Toxicity

Animals. Twelve male and female mongrel dogs, 19–25 kg in weight, were conditioned for 2 weeks in the Research Animal Facility at the University of Minnesota prior to study. Baseline serum creatinine, liver function tests, and complete blood count were determined on all dogs before CSA or vehicle administration. Table I summarizes the sequential administration of drug formulations to each dog. Successive studies on the same animal were conducted at least 1 week apart following reconfirmation of normal laboratory values and satisfactory physical examination by a veterinarian. In each study, a 16-gauge intravenous catheter was placed in a foreleg cephalic vein while the dog was manually restrained. After demonstrating free withdrawal of blood and injection of 10 ml saline without evidence of infiltration, the freshly prepared drug formulation was drawn up into a syringe and manually infused over the time intervals indicated below. The catheter was then flushed with 5 ml saline to dislodge any residual drug and removed.

Sandimmune I.V. Sandimmune I.V. was administered as a single intravenous bolus over 0.5 min to dogs 1 and 2 at doses of 14.0 and 12.5 mg/kg, respectively. The occurrence of severe reactions in these two dogs immediately following completion of the bolus necessitated a dose reduction and prolongation of infusion time in dogs 3 and 4. Dog 3 received 2.2 mg/kg over 5 min, at which time infusion was stopped due to the development of severe toxicity. In dog 4, infusion of 1.1 mg/kg over 30 min was not interrupted despite the occurrence of a persistent, more mild reaction.

CSA/Emulsion, CSA/Alcohol, and CSA/Liposomes. CSA, 2.5 mg/kg (4–6 ml of each 10 mg/ml preparation), was administered as a single intravenous dose over 5 min via a leg vein. Two dogs received CSA/emulsion (dogs 4 and 5), seven dogs received CSA/alcohol (dogs 5–11), and eight dogs received CSA/liposomes (dogs 1, 3, and 7–12).

Table I. Summary of Administration of CSA Formulations to 12 Mongrel Dogs

Dog No.	Sandimmune I.V.	CSA/emulsion	CSA/alcohol	CSA/liposomes	Emulsion vehicle	Ethanol vehicle
1	+	−	−	+	+	−
2	+	−	−	−	−	−
3	+	−	−	+	+	−
4	+	+	−	−	−	−
5	−	+	+	−	−	+
6	−	−	+	−	−	+
7	−	−	+	+	−	+
8	−	−	+	+	−	+
9	−	−	+	+	−	+
10	−	−	+	+	−	−
11	−	−	+	+	−	−
12	−	−	−	+	−	−

Vehicle Controls. Equivalent volumes (4–6 ml, according to body weight) of the "blank" emulsion vehicle and 47.5% ethanol/saline vehicle (both without added CSA) were administered to two dogs (dogs 1 and 3) and five dogs (dogs 5–9), respectively, as a single intravenous dose over 5 min via a leg vein. These animals were closely observed for drug reactions and toxicity during the first hour postdose.

Whole-Blood CSA Concentrations. Following the completion of each intravenous CSA administration, blood samples were drawn from the jugular vein in heparinized syringes at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 24, 28, 34, and 48 hr postdose. Each blood sample (2 ml) was placed in a calcium-EDTA blood collecting tube and refrigerated until CSA assay by high-performance liquid chromatography (23). The lower limit of sensitivity of the assay was 25 ng/ml. All CSA blood concentration–time data were analyzed by a noncompartmental method described by Chan *et al.* (24).

Pump Compatibility Studies

The components, operation, and programming of the Synchromed infusion pump (Model 8610H, Medtronic, Inc., Minneapolis, Minn.) used in our canine renal allograft model have been previously described (2). The use of a particular drug and vehicle in the peristaltic pump/catheter system depends on their compatibility with the material of the pump and catheter tubing. The linear dimensions of the tubing are critical to the smooth operation of the Synchromed pump, since changes in length as small as 0.2% will eventually result in gathering of the tubing and stalling of the device. We have found that significant changes in the weight and tensile strength of the silicone elastomer of the pump tubing (reflecting changes in linear dimensions) are predictive of pump malfunction, and we therefore examined these properties during exposure of the material to the emulsion and liposomal vehicle preparations (without added CSA) for 14 days. Five strips of silicone rubber (Medtronic, Inc.) were submerged into vials containing one of the two vehicles or a PBS control, and all the vials were maintained at 37°C. On days 4, 8, and 14, the strips were removed from each preparation and blotted dry prior to weighing and tensile strength testing. The weight and tensile strength of five additional untreated (day 0) samples were also determined. The ultimate tensile strength (tensile strength at break point) of each sample was measured using the Instron tensile tester (Model 1130, Instron Corporation, Park Ridge, Ill.).

Statistical Analysis

Mean percentage weight gain and ultimate tensile strength of the silicone samples were compared between groups in the pump compatibility studies using a nonpaired Student's t test. The total-body clearances of CSA/alcohol and CSA/liposomes in dogs receiving both preparations (dogs 7–11) were compared using a paired Student's t test. A P value ≤ 0.05 was regarded as statistically significant.

RESULTS

Acute Toxicity

Sandimmune I.V. Dogs 1 and 2 demonstrated severe reactions immediately following intravenous bolus administration, while infusion was terminated in dog 3 due to the development of severe toxicity. Vomitting, diarrhea, and seizure activity were pronounced in all three animals, despite the marked dose reduction and longer interval of infusion in dog 3. In dogs 1 and 3, the reaction completely resolved within 1 hr, although dog 1 had bloody stools for 3 days following the study. Dog 2, however, developed gastrointestinal hemorrhage with persistent hypotension refractory to intravenous fluid administration and required sacrifice 6 hr postdose. Dog 4 exhibited a milder reaction, including head-shaking, sneezing, and tachypnea, throughout the 30 min of drug delivery. Since significant reactions were observed with slowly administered doses of Sandimmune I.V. as low as 1–2.5 mg/kg, our initial protocol was amended so that a dose of 2.5 mg/kg of each subsequently studied CSA preparation was infused within a 5-min period.

CSA/Emulsion. Dog 4 exhibited a moderately severe reaction, with vomitting, diarrhea, and tachypnea lasting 45 min postdose. Dog 5 tolerated the preparation without incident.

CSA/Alcohol. Of the seven dogs receiving CSA/alcohol, five exhibited immediate mild reactions of head-shaking and sneezing (dogs 5–7, 9, and 10). In addition, dogs 5 and 6 were incontinent of stool and appeared sluggish for several hours, while dog 10 vomitted twice at 30 min and 1 hr

Table II. Pharmacokinetics of Intravenous Bolus Administration of Sandimmune I.V. to Three Dogs and CSA/Emulsion to Two Dogs[a]

	Dog No.	$T_{1/2}$ (hr)	K (L/hr)	AUC (hr · ng/ml/kg)	AUMC (hr^2 · ng/ml/kg)	MRT (hr)	V_{ss} (ml/kg)	TBC (ml/kg/min)
A	1	8.92	0.078	1264	17409	13.78	7446	9.01
	3	3.11	0.223	92	470	5.09	6231	20.38
	4	3.65	0.190	92	540	5.86	3037	8.64
B	4	7.67	0.090	381	4178	10.96	3125	4.75
	5	11.36	0.061	246	4436	18.05	7347	6.78

[a] Sandimmune I.V. was administered as described in the text. Dog 2 required sacrifice 6 hr after receiving Sandimmune I.V. and was excluded from analysis. CSA/emulsion (2.5 mg/kg) was given as a single intravenous dose over 5 min via leg vein.

postdose. Two dogs (dogs 8 and 9) tolerated the preparation without apparent side effects.

CSA/Liposomes. No discernible reactions occurred in any of the eight dogs receiving this preparation.

Vehicle Controls. Dogs 1 and 3 became bradycardiac, ataxic, and incontinent immediately following bolus administration of the "blank" emulsion preparation. Intravenous fluid administration was begun, and the animals remained sluggish and did not fully recover until 30 min postdose. All five dogs receiving the 47.5% ethanol/saline vehicle exhibited immediate mild reactions of head-shaking and sneezing, identical to those occurring in the majority of dogs receiving CSA/alcohol. These findings suggest that the toxicity observed following CSA/emulsion and CSA/alcohol administration was due predominantly to the vehicle, and not to the CSA.

Intravenous Pharmacokinetics.

Sandimmune I.V. and CSA/Emulsion. Tables IIA and IIB give the elimination half-life ($T_{1/2}$), elimination rate constant (K), area under the curve (AUC), area under the moment curve (AUMC), mean residence time (MRT), volume of distribution at steady state (V_{ss}), and total-body clearance (TBC) for the three surviving dogs (dogs 1, 3, and 4) receiving Sandimmune I.V. and the two dogs receiving CSA/emulsion (dogs 4 and 5).

CSA/Alcohol and CSA/Liposomes. Tables III and IV characterize the pharmacokinetics of intravenous bolus administration of CSA/alcohol ($N = 7$) and CSA/liposomes ($N = 8$), respectively. For each preparation, there is a three- to fourfold individual variation in $T_{1/2}$ and TBC among the animals studied. The CSA blood concentration versus time plot for one of the five dogs receiving both preparations (dog 10) is shown in Fig. 1, illustrating the striking similarity between the disposition of CSA/alcohol and that of CSA/liposomes seen in all five of these animals (dogs 7–11). There is no significant difference between the two preparations with regard to mean TBC in these five animals ($P = 0.6$), with four of the five dogs possessing equivalent clearance values and one with TBC (CSA/liposomes) > TBC (CSA/alcohol).

Pump Compatibility Studies

Figures 2A and 2B depict the time-dependent effect of PBS, emulsion, and liposomal vehicle incubation on the weight and tensile strength of the silicone pump tubing material, respectively. There was no significant effect of the PBS control solution on sample weight or tensile strength over the 2-week incubation period. Compared to the PBS control, the liposomal preparation produced a statistically significant decrease in mean tensile strength on day 4 only (1403 *vs* 1341 psi; $P = 0.02$) and a significant increase in mean percentage weight gain on days 4 (0.07 *vs* 0.31%; $P = 0.03$), 8 (0.04 *vs* 0.29%; $P = 0.04$), and 14 (0.12 *vs* 1.19%; $P < 0.0005$). However, these effects were small and did not reach the point at which pump function would be impaired (ultimate tensile strength below 1100 psi and weight gain above 5%; unpublished data). In contrast, the emulsion vehicle produced an early (day 4) weight gain of 22% and a 33% decrease in tensile strength to 944 ± 94 (SD) psi, with no further change on continuing exposure.

DISCUSSION

Following the introduction of CSA in 1980, CSA-

Table III. Pharmacokinetics of Intravenous Bolus Administration of CSA/Alcohol (2.5 mg/kg) to Seven Dogs[a]

Dog No.	Weight (kg)	$T_{1/2}$ (hr)	K (L/hr)	AUC (hr · ng/ml/kg)	AUMC (hr^2 · ng/ml/kg)	MRT (hr)	V_{ss} (ml/kg)	TBC (ml/kg/min)
5	25.0	10.01	0.069	331	5204	15.72	4748	5.03
6	20.0	5.13	0.135	225	1684	7.47	4146	9.25
7	22.5	5.95	0.117	191	1738	9.08	5273	9.68
8	20.2	11.21	0.062	445	8258	18.57	5167	4.64
9	20.5	3.19	0.217	92	462	5.02	6664	22.11
10	21.5	4.26	0.163	179	1187	6.64	4317	10.84
11	20.2	7.44	0.093	270	3151	11.69	5315	7.58
Mean	21.4	5.67	0.122	248	3098	10.60	5090	7.82
SD	1.8	2.98	0.055	115	2754	5.00	834	5.88

[a] Harmonic means are given for $T_{1/2}$ and TBC. All other mean values are arithmetic.

Table IV. Pharmacokinetics of Intravenous Bolus Administration of CSA/Liposomes (2.5 mg/kg) to Eight Dogs[a]

No. Dog	Weight (kg)	$T_{1/2}$ (hr)	K (L/hr)	AUC (hr · ng/ml/kg)	AUMC (hr^2 · ng/ml/kg)	MRT (hr)	V_{ss} (ml/kg)	TBC (ml/kg/min)
1	20.5	4.26	0.163	226	1477	6.54	3531	9.00
3	19.5	4.51	0.154	170	1239	7.27	5465	12.53
7	21.6	3.64	0.190	137	810	5.90	4972	14.05
8	20.2	10.71	0.065	398	6708	16.88	5254	5.19
9	20.5	5.29	0.131	107	1001	9.33	10598	18.93
10	21.5	4.59	0.151	162	1191	7.35	5273	11.96
11	20.0	6.72	0.103	258	2591	10.04	4864	8.07
12	21.4	5.95	0.116	167	1564	9.39	6589	11.69
Mean	20.7	5.16	0.134	203	2073	9.09	5858	9.99
SD	0.8	2.25	0.039	92	1949	3.48	2106	4.15

[a] Harmonic means are given for $T_{1/2}$ and TBC. All other mean values are arithmetic.

containing drug regimens have markedly improved graft and patient survival rates of allograft recipients over those achievable with conventional immunosuppression (25,26) and have now become the mainstay of antirejection therapy in organ transplantation. However, CSA-induced nephrotoxicity continues to be a major factor limiting the usefulness of the drug in general (27,28), and administration of the currently available intravenous formulation, Sandimmune I.V., appears to be particularly associated with adverse systemic reactions, including anaphylaxis (10–14) and adult respiratory distress syndrome (15). This formulation combines two potentially toxic agents whose effects may be additive in certain circumstances: free lipophilic drug and the solubilizing carrier, Cremophor EL.

Cremophor EL (polyethylenglycolglycerol ricinoleate) has been used to dissolve a number of hydrophobic drugs for intravenous administration in addition to CSA, including the anesthetics Althesin, propanidid and 2,6-diisopropylphenol, and diazepam (4–6,9). There have been several reports of anaphylactic reactions to these Cremophor EL formulations in both canine and porcine animal models and in man (4–7,29). All four dogs receiving Sandimmune I.V. in our study exhibited significant adverse reactions following intravenous administration despite progressive reduction in dose from 14 to 1.1 mg/kg and prolongation of infusion time from 0.5 to 30 min, with one animal requiring sacrifice 6 hr postdose. Although the acute toxicity we observed was quite pronounced and consistent with the extensive canine studies of Lorenz *et al.* (7), it is interesting to note that no mention of adverse reactions to Sandimmune I.V. appeared in any of the following three recent reports on CSA disposition in the dog in which similar or higher doses were given: (1) Gridelli *et al.* (30) (5 mg/kg as a bolus), (2) White *et al.* (31) (16 mg/kg over 1 min), and (3) Buice *et al.* (32) (20 mg/kg over 30 min).

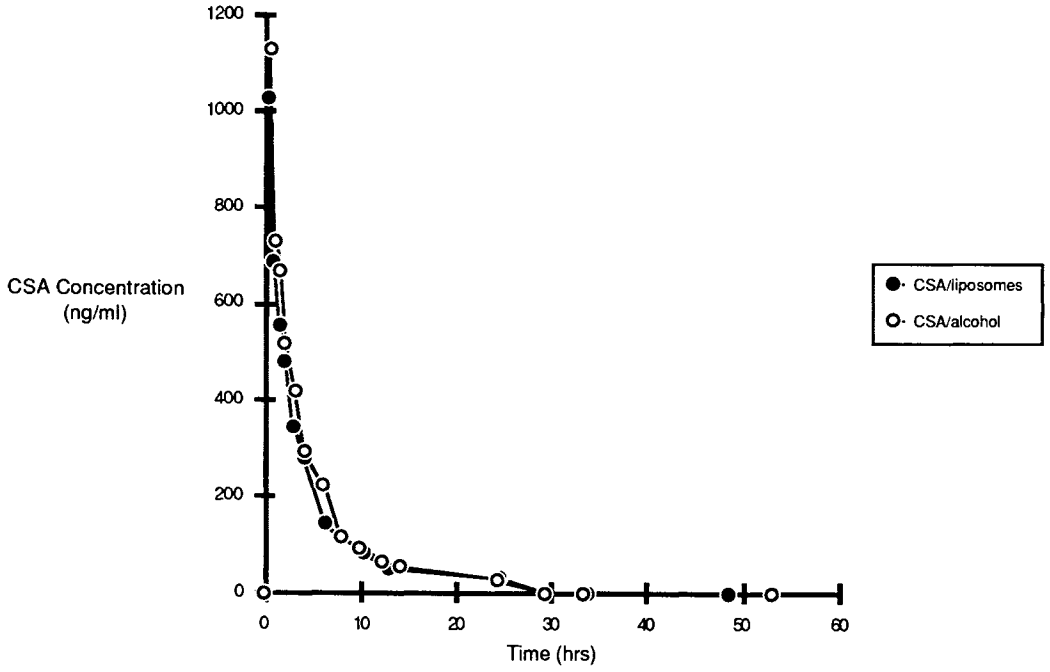

Fig. 1. CSA blood concentration *vs* time plot following a single intravenous dose of 2.5 mg/kg CSA/liposomes (filled circles) and CSA/alcohol (open circles) administered to dog 10 over 5 min in sequential studies. The overlapping curves demonstrate a similar disposition for these two CSA formulations.

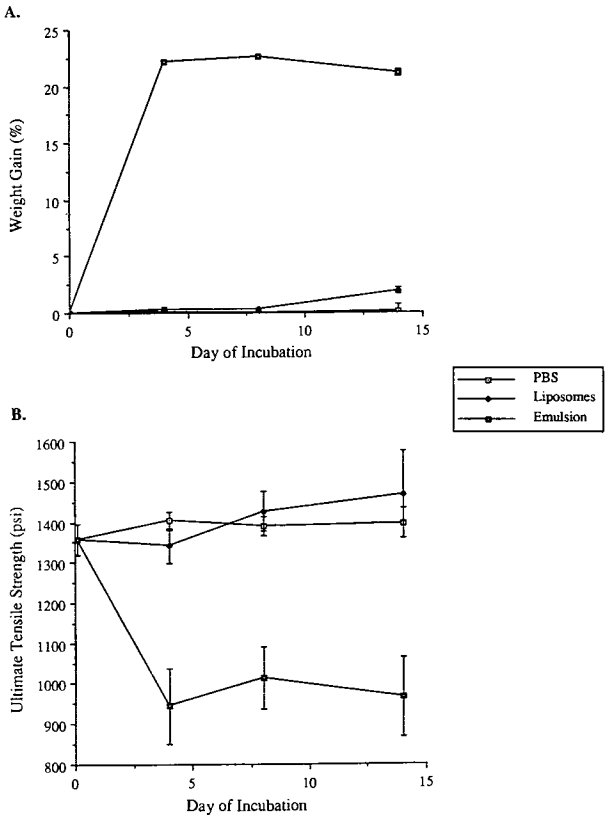

Fig. 2. Percentage weight gain (A) and ultimate tensile strength (B) of silicone pump tubing material during 2-week exposure to emulsion vehicle, liposomal vehicle, and PBS control. Values plotted are means ± SD of five silicone rubber strips. In contrast to the liposomal vehicle, the emulsion vehicle produced a marked, early increase in tubing weight and decrease in tensile strength, which would adversely affect pump delivery rates.

Gridelli *et al.* (30) reported a systemic clearance of 7.06 ± 0.87 (SE) ml/min/kg after Sandimmune I.V. administration to five dogs when CSA concentrations were determined by high-performance liquid chromatography. These values were similar to those obtained in dogs 1 and 4 in our study. However, in view of the severe reactions elicited by Sandimmune I.V. in our animals, interpretation of the pharmacokinetic data in Table IIA must be guarded, and we found it necessary to use another, less toxic CSA preparation as a "control" (CSA/alcohol) with which to compare legitimately the disposition of the "test" liposomal formulation.

One approach which has been taken to circumvent the untoward effects of Cremophor EL formulations of fat-soluble substances is the use of an emulsion vehicle. Glen and Hunter (9) reported that a new emulsion formulation of 2,6-diisopropylphenol has anesthetic properties in rats and mice and hemodynamic effects in the minipig similar to those of a previously available Cremophor EL formulation. This emulsion, however, does not produce marked increases in plasma histamine concentration on administration to dogs and anaphylactoid responses on second injection in minipigs. In contrast to the experience of Huttel *et al.* (5) with Stesolid MR (diazepam with Cremophor EL as solvent), von Dardel *et al.* (18) found that an emulsion preparation of diazepam was well tolerated in 2435 patients, with only 9 patients experiencing pain on intravenous injection, 26 patients demonstrating an unsatisfactory clinical effect, and 1 patient developing transient erythema. Despite these successes, our initial attempt to develop an emulsified CSA preparation was disappointing with both dogs receiving vehicle alone and one of the two animals receiving CSA/emulsion demonstrating moderately severe, although transient, reactions. As a result, this formulation, like Sandimmune I.V., could not be used as a control for pharmacokinetic analysis of CSA/liposomes. Furthermore, the "blank" emulsion produced early, marked changes in the weight and tensile properties of the pump tubing material during the 14-day incubation period and is, therefore, incompatible with the SynchroMed infusion pump in its present form. We are currently testing the individual components of the emulsion vehicle, alone and in various combinations and proportions, in acute toxicity and pump compatibility studies in order to develop a nontoxic, pump-compatible, readily available, and relatively inexpensive parenteral CSA preparation for systemic and local infusion.

Incorporation of hydrophobic drugs within liposomes not only represents a second feasible approach for eliminating the toxic Cremophor EL solvent, but also might permit one to alter the pharmacokinetics (33,34) and reduce the acute and chronic organ- and cell-specific toxicity of the free drug (35,36). Since the initial observation by Aziz *et al.* (21) that low doses of parenterally administered CSA-miglyol liposomes were effective in prolonging rat cardiac allograft survival without significant systemic toxicity, further studies have addressed the reduction of CSA and/or Cremophor EL-induced nephrotoxicity. In a rat model, Hsieh *et al.* (19) measured a significantly greater decrease in glomerular filtration rate after 2 weeks of daily Sandimmune I.V. administration (12.5 mg/kg) than with infusion of twice the dose of liposome-encapsulated CSA. More recently, Smeesters *et al.* (20) demonstrated that unilaterally nephrectomized rats treated with a CSA-liposome preparation (25 mg/kg for 14 days) had better survival rates, less weight loss, and lower serum creatinine values than those treated with Sandimmune I.V.

Our liposomal CSA formulation produced no gross evidence of systemic toxicity following a single intravenous dose and displayed pharmacokinetic parameters equivalent to that of the CSA/alcohol control. Unlike solvents containing a high percentage of ethanol, the liposomal vehicle did not adversely effect the silicone elastomer of the pump tubing in our compatibility study and appears to be a suitable formulation for short-term intrarenal administration. Furthermore, by changing the size of the liposomal carriers, the systemic disposition of free drug could conceivably be altered to increase the pharmacokinetic advantage of local CSA infusion by increasing renal extraction or systemic clearance (3). Finally, we have developed a freeze-dried liposomal preparation which has demonstrated long-term stability and sterility with maintenance of vesicle size and percentage CSA incorporation (37). We would next like to determine the long-term effects of different doses of CSA/liposomes on (i) renal function, (ii) renal morphology, (iii) systemic drug concentrations, and (iv) systemic toxicity in dogs undergoing renal autotransplantation. In addition, compatibility of the liposomal preparation with the

pump components over longer periods of time must be ascertained.

In conclusion, our preliminary studies describe the first investigation of the pharmacokinetics and acute toxicity of a liposomal CSA preparation in a large animal model. This work may be of importance to the successful development of a much-needed, less toxic parenteral CSA formulation. It also represents a foundational step in the design of future studies utilizing intrarenal liposomal CSA delivery by an implantable pump to achieve local immunsuppression of kidney transplants with diminished renal and systemic toxicity.

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