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Liposomal Encapsulation of Foscarnet Protects against Hypocalcemia Induced by Free Foscarnet

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Hypocalcemia and an increase in creatinine level are the most important serious effects associated with foscarnet (PFA) therapy. In an animal model, we have explored the potential protective role of liposome-encapsulated foscarnet (LE-PFA) on these metabolic abnormalities. PFA administered as one bolus injection (0.5 or 1.0 g/kg) caused significant rapid decreases (~20%) in the levels of calcium and phosphorus in serum within a few minutes and up to 30 min after injection. LE-PFA did not induce any of these changes, while peak levels in serum and the half-life of this formulation were much higher than those of the free drug. PFA administered for 2 weeks (340 or 500 mg/kg/day) resulted in no changes in creatinine or blood urea nitrogen levels in serum at the low-dosage level, but at the higher-dosage level, the creatinine level in serum increased by day 5 posttreatment. Furthermore, there was no increase in the creatinine or blood urea nitrogen level after 2 weeks of treatment with LE-PFA at a dosage of 35 mg/kg/day. When the pharmacokinetics of both free PFA and LE-PFA were compared, the plasma half-life of the encapsulated drug was ~four times longer than that of the free drug. In addition, the systemic clearance of LE-PFA was ~one-fifth of that of the free drug. In conclusion, free PFA causes hypocalcemia and hypophosphatemia and increases the creatinine level in serum, whereas the LE form of this drug seems to protect against the abnormal changes in calcium and phosphorus levels caused by the free drug. By preventing hypocalcemia and increasing its half-life, LE-PFA can be used at lower doses and at longer intervals. Clinical investigations of these formulations may be worthwhile.

Foscarnet (PFA) is a pyrophosphate analog which prevents the replication of human herpesviruses, including cytomegalovirus (CMV), by inhibiting viral DNA polymerases (23). PFA also inhibits human immunodeficiency virus reverse transcriptase (13, 15, 32). Combined with zidovudine (AZT), PFA is synergistic against human immunodeficiency virus (10). This drug has been approved for the treatment of CMV retinitis in patients with AIDS (5, 16). CMV infections are frequent in immunosuppressed patients, notably those with chemotherapy-induced suppression, such as transplant recipients and patients with AIDS. In these patients, CMV infection often becomes a fatal disease, whereas in immunocompetent adults, it is usually of minor importance.

Both continuous and intermittent intravenous infusions have been investigated as induction therapy, with the latter approach now preferred. Induction therapy starts with an intravenous bolus injection of PFA (20 or 30 mg/kg), followed by a continuous infusion of 230 mg/kg/day for 2 to 4 weeks. Continuous infusion has largely been superseded by the alternative induction regimen of infusion of PFA at 60 mg/kg every 8 h or 90 to 100 mg/kg every 12 h for 2 to 4 weeks (5). Because relapse occurs in approximately 66% of surviving patients within a month of stopping induction therapy, subsequent maintenance treatment is given. Over 60% of patients receiving maintenance schedules of 2-h intravenous infusions of PFA at 60 to 90 mg/kg/day for 5 days each week have had relapses within 2 to 4 weeks. In contrast, the administration of PFA at 90 to 120 mg/kg each day delayed retinitis progression for 17 to 23 weeks on average. In human immunodeficiency virus-infected patients, 83% of a cumulative intravenous dose of PFA was recovered unchanged in urine during the first 36 h of stopping the infusion (5).

A major problem associated with the administration of PFA remains its marked toxicity. The most frequently reported major adverse effect associated with PFA therapy is nephrotoxicity. Elevations in the creatinine levels in serum occur in ~45% of patients; hemodialysis may be required in severe cases of renal impairment (4, 5, 7). Reversible calcium and phosphorus abnormalities occur in ~66% of PFA recipients (14, 16, 25). No metabolites of PFA have been detected. The encapsulation of drugs into liposomes is an interesting approach to reduce their toxic effects. Several studies have shown improved therapeutic indices and reduced toxicities for drugs encapsulated in liposomes (2, 17, 21). Among them and of particular relevance to our work are antiviral agents. It was shown that liposome-encapsulated (LE) AZT resulted in decreased bone marrow toxicity and enhanced activity against murine AIDS (27, 28). It was also shown that LE-ganciclovir and LE-PFA had greater anti-CMV activities in human embryonic lung fibroblast cells (3). Furthermore, LE-PFA was shown to be more effective (in Vero cell tissue culture) against herpes simplex virus (type 2) replication than was the free drug, without any increase in cytotoxicity (34).

Because most of the toxicity data of PFA comes from clinical trials and there is hardly any data about the toxicity of this drug in animal models, we conducted experiments to establish and better understand PFA toxicity in mice (C57BL/6). The advantage of such a study is twofold. First, we can evaluate and compare the toxicities of free PFA and LE-PFA. Second, this strain of mice can be infected with murine CMV and murine retrovirus inducing murine AIDS, a situation very close to that of patients with AIDS who are infected with CMV, thus making it an excellent model for studying drug toxicity and efficacy.

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MATERIALS AND METHODS

Chemicals. PFA was purchased from Sigma Chemical Company, St. Louis, Mo.; dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) were purchased from Avanti Polar Lipids, Pelham, Ala.; [^{3}H]DPPC and [^{14}C]PFA were obtained from Amersham Canada, Ltd., Oakville, Ontario, Canada, and Moravek Biochemical, Inc., Brea, Calif., respectively.

LE form of PFA: preparation and characterization. Liposomes were prepared by the reverse-phase evaporation technique as described previously (29, 33). Briefly, 66 μmol of a lipid mixture of DPPC and DPPG at a molar ratio of 10:3 was dissolved in 3 ml of isopropyl ether–0.8 ml of methanol for better solubilization of synthetic lipids. To this organic phase, 1 ml of an aqueous phase (iso-osmotic phosphate-buffered solution [PBS; pH 7.4]) containing PFA at a drug/lipid molar ratio of 5:1 was added. This mixture was homogenized by sonication for 5 min in an ultrasonic bath (Branson model 2200; Fisher Scientific, Montréal, Québec, Canada), and the organic solvent was evaporated under vacuum at 50°C. For toxicity experiments, [^{3}H]DPPC (2 μCi; <0.001% μmol/μmol of cold DPPC) and [^{14}C]PFA (1 μCi; 0.003% μmol/μmol of cold PFA) were used as tracers for liposomes and drug, respectively; five times more radioactivity was used for pharmacokinetics experiments. Liposomes were then extruded with a stainless steel extrusion device (Lipex Biomembrane, Vancouver, British Columbia, Canada) through polycarbonate membranes (Nucleopore, Cambridge, Mass.) with pore diameters of 0.5 and 0.1 μm to generate liposomes with diameters of 107 ± 10 nm. Vesicle size distribution and homogeneity were evaluated by quasielastic light scattering in a submicron particle analyzer (model N4SD; Coulter Electronics, Hialeah, Fla.). The entrapment efficacy was determined after the separation of free PFA and LE-PFA by centrifugation at 300 × *g* for 15 min (twice) at 4°C of liposomes (1 ml) through a 10-ml coarse Sephadex G-50 column (Pharmacia LKB, Montréal, Québec, Canada). The concentrations of encapsulated drug and liposomal phospholipids were determined by counting radioactivity with a liquid scintillation counter (model LS 6000TA; Beckman Instruments Canada, Inc., Mississauga, Ontario, Canada), with [^{3}H]DPPC and [^{14}C]PFA as radioactive tracers.

Drug retention was evaluated in vitro after the incubation of liposomes with PBS containing 10% fetal bovine serum (FBS), FBS, or PBS at 37°C or with PBS at 4°C. At each time point, 0.1 ml of liposome solution was removed and centrifuged through a 1-ml Sephadex G-50 column (300 × *g* for 5 min at 4°C). Drug retention was evaluated by radioactivity counting of eluates and calculated with respect to that at time zero, which was regarded as 100%.

Animals and treatment for toxicity studies. Experiments were performed on female C57BL/6 mice (Charles River Breeding Laboratories, Inc., Montréal, Québec, Canada) weighing 18 to 20 g. Animals were maintained in an animal facility with a light-dark cycle of 14 and 10 h, respectively (light on at 0600 h), and had free access to water and standard mice chow. Animals were injected with saline (control group), PFA, or LE-PFA. PFA solution (in PBS with the pH adjusted to 7.2) was injected (in an ~0.3-ml volume) intraperitoneally (i.p.) three times a day. In one experiment, the PFA total dosage was 500 mg/kg/day for 17 days. In another experiment, about two-thirds of the above dosage was used (340 mg/kg/day for 15 days); LE-PFA was injected once daily at a dose of 35 mg/kg for 15 days (this is the maximum LE-PFA dose that could be used because of the limit of encapsulation). In a different set of experiments to determine the effects of PFA on calcium and phosphorus levels in serum, animals were treated with one injection of PFA i.p. either at 1 g/kg (in a 0.5-ml volume) for the calcium and phosphorus time course experiment or at different concentrations (from 0 to 1 g/kg) for the PFA dose course experiment. Once an optimum time point and optimum dosage for PFA effects on calcium and phosphorus were selected, a last group of experiments were carried out to compare the effects of free PFA and LE-PFA (at the same dose level [0.5 g/kg in a 1-ml volume]). In these experiments, as we wanted to inject a maximal volume of 1 ml, LE-PFA had to be concentrated in an ultrafiltration-stirred cell (10 ml) (Amicon, Inc., Beverly, Mass.) by using membranes (YC05) with a molecular weight cutoff of 500. Our preparations of LE-PFA give drug concentrations of between 3.6 and 7.0 mg/ml of liposomes. For a preparation of LE-PFA with a PFA concentration of 5.0 mg/ml, to inject 500 mg/kg into a 20-g mouse, it is necessary to inject a volume of 2 ml, which is double the maximum volume that can be injected i.p. Therefore, we had to concentrate the liposomal preparation. This was successfully achieved by using an ultrafiltration-stirred cell with membranes having a molecular weight cutoff of 500. Depending on the initial drug concentration in liposomes, liposomes can be concentrated severalfold; we tried up to a fourfold maximum with good stability. Drug retention was judged by counting radioactivity after the centrifugation of liposomes on a Sephadex G-50 column to separate free drug from encapsulated drug. Moreover, liposomal size and appearance remained unchanged after being concentrated.

Sampling and sacrifice. Blood samples were collected at different times throughout each experiment (see figure legends for sampling times) from the retro-orbital plexus (at the corner of the eye) in Eppendorf tubes to obtain serum samples for biochemical analyses. The levels of creatinine, blood urea nitrogen (BUN), glutamate pyruvate transaminase (also called alanine aminotransferase), and total calcium and phosphorus in serum samples were determined by automated assays with a Hitachi 737 autoanalyzer and kits from Boehringer Mannheim in the clinical biochemistry laboratory at the Centre Hospitalier de l'Université Laval.

Pharmacokinetic evaluation. Another group of mice received one bolus i.p. dose of either free PFA or LE-PFA at the same dose level (75 mg/kg), and blood samples were collected from the retro-orbital plexus of each mouse at different times after treatment (0 to 4 h for free drug and 0 to 24 h for LE-PFA). The pharmacokinetic parameters for each mouse were determined by using a noncompartmental model. The elimination rate constant was estimated (by using a computer package supplied kindly by J. Turgeon of the School of Pharmacy at Laval University) from the slope of the terminal elimination phase of the plasma concentration time curve. The half-life in plasma of the terminal elimination phase ($t_{1/2}$) was calculated as 0.693/elimination rate constant. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) and the area under the first moment of the concentration-time curve were calculated by using the log-trapezoidal rule with the terminal portion extrapolated to infinity. The systemic clearance was calculated as dose/$AUC_{0-\infty}$, while the mean residence time was calculated as the area under the first moment of the concentration-time curve/$AUC_{0-\infty}$. The maximum drug concentration in plasma (C_{max}) and the time when C_{max} was obtained were evaluated by visual inspection of the results for each mouse.

Statistical analysis. All statistical analyses were performed with Stat View SE + Graphics (Abacus Concepts, Inc., Berkeley, Calif.). The differences between groups were tested by analysis of variance (ANOVA). Group comparisons were performed by using Fisher's PLSD test, and a difference with a $P < 0.05$ was considered to be statistically significant (P values are given in the figure legends).

RESULTS

The toxicities of PFA and LE-PFA on calcium and phosphorus in serum were compared. Figure 1A shows the influence of free PFA (1 gm/kg [i.p.]) on calcium levels in serum at different times (0 to 120 min) after one bolus i.p. injection. There was a significant rapid decrease in the calcium level up to 30 min (20% decrease); thereafter, it started to increase slowly, presumably because of rapid elimination of the drug by the kidney. Phosphorus levels in serum behaved similarly (Fig. 1B). On the basis of the previous experiment, we chose 30 min as the optimal time to determine the lowest dose (between 0 and 1 g/kg) that induced changes in serum levels of calcium and phosphorus. PFA doses of 0.5 and 1 g/kg significantly decreased calcium levels (Fig. 2A). There were dose-dependent decreases in the phosphorus levels in serum, with the lowest level (45% decrease) obtained at the highest dose of PFA (Fig. 2B).

Once the optimum dose and time for the free drug were determined, PFA was entrapped in liposomes to study the effects of encapsulation on drug toxicity. Figure 3 shows the retention of PFA in liposomes. The release of less than 30% of [^{14}C]PFA was observed when LE-PFA was incubated for 48 h at 37°C in PBS containing 10% FBS or in FBS, whereas no leakage of drug was noticed when LE-PFA was kept in PBS at 4 or 37°C for 48 h. Analysis revealed that LE-PFA had a reproducible encapsulation efficiency of 0.26 ± 0.05 μmol of PFA per μmol of lipids into liposomes with an average diameter of 107 ± 10 nm.

The pharmacokinetic parameters of free PFA and LE-PFA are given in Table 1. The free drug had a very short $t_{1/2}$ of 0.7 h, whereas LE-PFA had a longer $t_{1/2}$ (2.5 h) and liposomes had a $t_{1/2}$ of ~8 h. The systemic clearance of the free form of this drug was five times higher than that of the encapsulated form. The time taken to reach C_{max} was 5 min for free PFA and 1 to 2 h for LE-PFA.

We then compared the effects of free PFA and LE-PFA on calcium and phosphorus levels in serum at 10 and 30 min after one bolus i.p. injection of 0.5 g/kg. Free PFA significantly decreased the calcium levels in serum at both 10 and 30 min after injection, whereas LE-PFA did not have any effect (Fig. 4A). The phosphorus levels in serum decreased at 10 min in the case of free PFA, but the difference was not significant at 30 min. LE-PFA did not have any effect on phosphorus levels in serum (Fig. 4B). Empty liposomes did not have any effect on

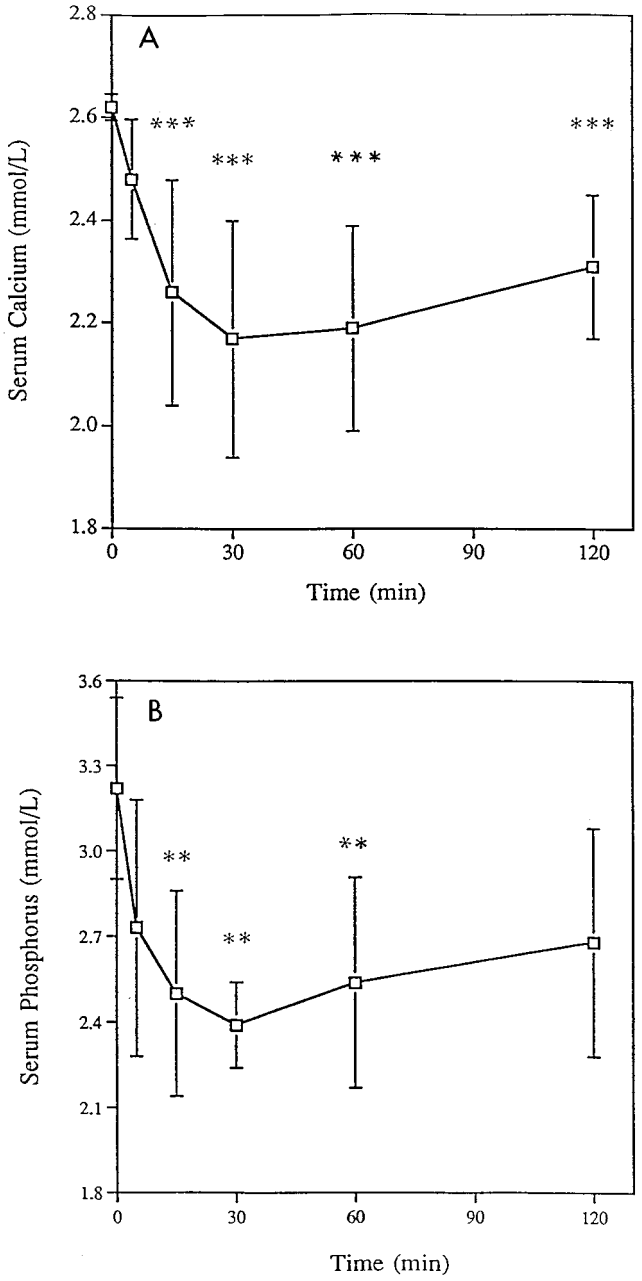

FIG. 1. Time course of the effects of PFA on the levels of calcium (A) and phosphorus (B) in serum. PFA (1 g/kg) was injected i.p. as one bolus dose, and blood samples were obtained from the retro-orbital plexus of each mouse at the time points indicated to obtain serum samples for calcium and phosphorus measurements. Data are the means ± standard deviations (SD) (n = six to nine mice per group). ** and ***, significant differences from value at time zero ($P <$ 0.01 and 0.001, respectively [by ANOVA]).

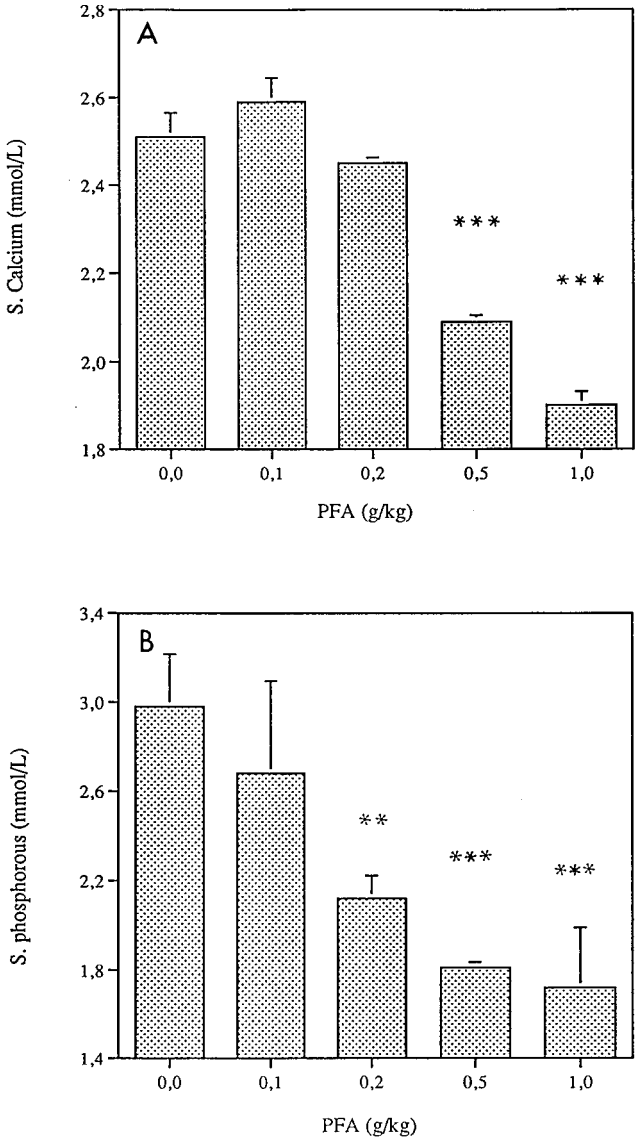

FIG. 2. Dose course of the effects of PFA on the levels of calcium (A) and phosphorus (B) in serum (S.). PFA (0 to 1 g/kg) was injected i.p. as one bolus dose, and blood samples were obtained from the retro-orbital plexus of each mouse at 30 min to obtain serum samples for calcium and phosphorus measurements. Data are the means ± SD (n = three to four mice per group). ** and ***, significant differences from value at dose zero ($P <$ 0.01 and 0.001, respectively [by ANOVA]).

the levels of either calcium or phosphorus in serum (data not shown).

In experiments concerning calcium and phosphorus measurements, we were able to test the same concentrations of free and encapsulated drug because there was only one bolus injection and the effects of PFA on calcium and phosphorus are transient and observed within minutes of injection. In case of nephrotoxicity, the creatinine levels in serum of patients treated with PFA usually start to increase within 5 to 7 days of treatment. Therefore, we carried out the nephrotoxicity experiments over a 2-week period. We tested a dosages of 340 mg/kg/day for 15 days for free PFA and 35 mg/kg/day for 15 days for LE-PFA (because it is impossible to encapsulate large quantities of PFA in liposomes). The level of neither creatinine nor BUN in serum increased 5, 10, or 15 days after injection. At the higher dosage of free PFA (500 mg/kg/day for 18 days), we observed a small but significant increase (~17% increase) in the level of creatinine in serum on day 5 after treatment, whereas the level of BUN did not change. This drug seems to be less nephrotoxic to mice than it is to humans. There were no changes in glutamate pyruvate transaminase levels in serum at either dosage level of free PFA or at the dosage of LE-PFA used (data not shown).

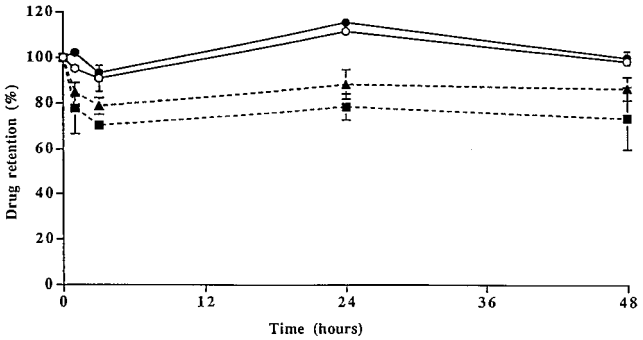

FIG. 3. Retention of PFA in liposomes (average diameter, 107 ± 10 nm) composed of DPPC-DPPG (molar ratio, 10:3). Drug retention was determined at both 4°C (open symbols) and 37°C (filled symbols) in PBS (circles), PBS–10% FBS (squares), and FBS (triangles). Each point represents the mean obtained from three experiments. Bars show SD.

DISCUSSION

The most frequent dose-limiting adverse effect of PFA therapy is impaired renal function. A two- to threefold increase in the levels of creatinine in serum has been observed in 20 to 66% of patients with AIDS who received 130 to 230 mg/kg/day of PFA as a continuous infusion (4, 5, 7); hemodialysis may be required in severe cases of renal impairment. This toxicity is believed to be due to acute tubular necrosis, which tends to be reversible on PFA withdrawal. Reversible calcium and phosphorus abnormalities (both increases and decreases were reported) have been observed in PFA recipients (14, 16, 25). PFA therapy is also associated with anemia in 20 to 50% of patients with AIDS (11, 12, 16). Another potentially treatment-limiting effect attributed to PFA is penile ulceration, which appears to result from exposure of the glans penis to unchanged PFA in urine (5, 14). PFA also causes thrombophlebitis of peripheral veins during continuous infusion (12). All of these adverse effects are based on clinical experience; none of them have been established in animal models, which, in turn, makes it very difficult to test new approaches, such as the LE form of the drug as a new delivery system. Therefore, in this study, we compared the toxicities of PFA and LE-PFA in mice.

To explore PFA toxicity in small animals is difficult. In our laboratory, we experienced some problems during the treatment of mice with PFA. At first, when we tried to inject PFA intravenously (1 g/kg once a day), there was inflammation and deterioration (thrombophlebitis) at the site of injection (the tail veins), even worse necrosis occurred, and finally the ulcerated part of the tail fell off within about 5 days of treatment. Furthermore, fast injection of PFA intravenously or the injection of a high dose (0.5 or 1 g/kg) causes seizures and cardiac arrest, resulting in the immediate death of the animal (within ~1 min). For these reasons, it was very difficult to test the toxicity of PFA in mice after intravenous injection(s) for periods of 2 or 3 weeks. Furthermore, it has been shown that liposomes reach blood circulation after i.p. injection; before appearing in blood, they have a biodistribution and pharmacokinetics similar to those of liposomes injected intravenously (1, 20). We chose the i.p. route of administration because there are fewer problems associated with it, even though we observed peritonitis (viscous fluid in peritone and/or edema) at the time of sacrifice (after about 2 weeks of treatment). Moreover, PFA injected subcutaneously caused cutaneous ulcerations with blood clots.

LE forms of drugs offer various advantages, improving drug

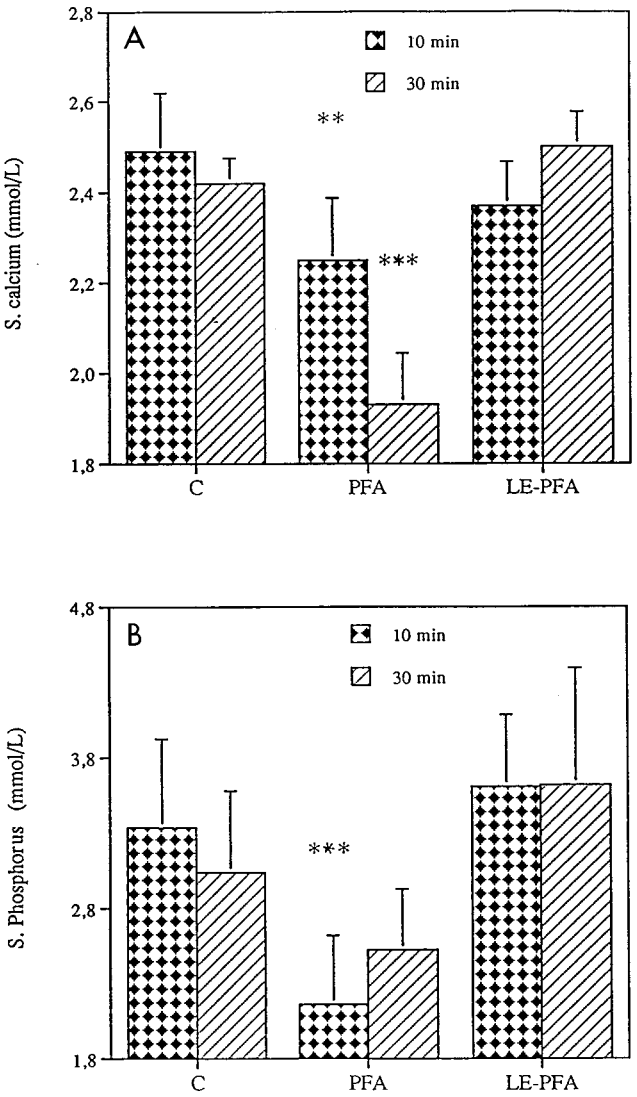

FIG. 4. Effects of free PFA and LE-PFA on the levels of calcium (A) and phosphorus (B) in serum. Both forms were injected once i.p. at a dose of 0.5 g/kg. Blood samples were collected from the retro-orbital plexus of each mouse at 10 and 30 min to obtain serum samples for calcium and phosphorus measurements. Data are the means ± SD (n = seven mice per group). ** and ***, significant difference from value for control group (C) ($P < 0.01$ and 0.001, respectively [by ANOVA]).

efficacy and pharmacokinetics, reducing toxicity, and targeting infection sites (for a review, see reference 18). Injected intravenously, liposomes tend to be rapidly taken up by phagocytic cells of the reticuloendothelial system, particularly by hepatic Kupffer cells and spleen macrophages. Many antibiotics (amphotericin B, gentamicin, ampicillin, cephalothin, and sisomicin) (2), antitumor agents (doxorubicin, cisplatin, dactinomycin, and others) (26), and more recently antiviral agents (ribavirin, AZT, dideoxyinosine, dideoxycytidine, and PFA) (3, 8, 19, 20, 27, 28) have been encapsulated into liposomes. Several studies have shown improved therapeutic indices and reduced toxicities for drugs encapsulated in liposomes. Among them, it was shown that LE-AZT resulted in decreased bone marrow toxicity and enhanced activity against murine AIDS (27, 28). It was also shown that LE-ganciclovir and LE-PFA had greater anti-CMV activities in human embryonic lung fi-

TABLE 1. Pharmacokinetics of free PFA and LE-PFA following i.p. administration of a single dose of 75 mg/kg

Parameter (unit)[a]	Value (mean ± SD) for[b]:		
	Free PFA ([^{14}C]PFA) (n = 4)	LE-PFA ([^{14}C]PFA) (n = 5)	LE-PFA ([^{3}H]DPPC) (n = 5)
$t_{1/2}$ (h)	0.71 ± 0.18	2.48 ± 0.56**	7.86 ± 1.10***,§§§
$AUC_{(0-\infty)}$ (nmol/ml/h)	424.70 ± 95.40	2,507.00 ± 994.00	22,698.00 ± 9,485.00***,§§§
k_{el} (h^{-1})	1.02 ± 0.24	0.29 ± 0.06***	0.09 ± 0.01***,§
Cl (liter/h/kg)	0.54 ± 0.13	0.10 ± 0.03***	0.01 ± 0.004***
MRT (h)	1.10 ± 0.28	4.46 ± 0.77**	13.11 ± 1.85***,§§§
T_{max} (h)	0.08 ± 0.00	1.40 ± 0.55***	2.00 ± 0.00***,§
C_{max} (nmol/ml of plasma)	564.10 ± 37.40	722.40 ± 251.00	3,472.00 ± 1,572.00***,§§§

[a] Parameters were calculated by using a noncompartmental model. k_{el}, elimination rate constant; Cl, total body clearance; MRT, mean residence time; T_{max}, time taken to reach C_{max} (both C_{max} and T_{max} were obtained by visual inspection of the results for each mouse).

[b] **, significantly different from value for free PFA ($P < 0.01$); ***, significantly different from value for free PFA ($P < 0.001$); §, significantly different from value for LE-PFA ([^{14}C]PFA) ($P < 0.05$); §§§, significantly different from value for LE-PFA ([^{14}C]PFA) ($P < 0.001$ [by ANOVA]).

broblast cells (3). Furthermore, LE-PFA was shown to be more effective against herpes simplex virus (type 2) replication than free PFA, without any increase in cytotoxicity (34). It was also demonstrated that the 50% lethal dose (LD_{50}) of amBisome (LE-amphotericin B) in mice was greater than 175 mg/kg (76-fold), compared with 2.3 mg/kg for conventional (free) amphotericin B (30). In rats, the LD_{50} of amBisome was 50 mg/kg (30-fold), compared with 1.6 mg/kg for the conventional drug. In general, these studies demonstrated increased therapeutic efficacy and reduced toxicity as a result of LE forms of drugs.

Hypocalcemia is the second (after the increase in creatinine) most important serious adverse effect associated with PFA therapy in patients. Both increased and decreased ionized and total calcium levels in serum have been observed in patients receiving PFA (16, 25); however, it appears that most clinical symptoms are related to decreases in calcium (ionized) levels associated with PFA administration (14). Hypocalcemia has been found to be associated with paresthesias and may account for tremors, arrhythmias, and seizures observed in patients receiving PFA, particularly in cases of acute overdose or too rapid infusion (16, 31, 35). Severe and even fatal total hypocalcemia has been reported for patients receiving PFA and parenteral pentamidine concomitantly (36). It was suggested that this effect may be produced by the complexing of PFA with ionized calcium, an explanation that was supported by the observation that phosphate, of which PFA is an analog, can complex with ionized calcium in solution (14). Our results show that i.p. injection of PFA into mice resulted in rapid decreases in the levels of both calcium and phosphorus in serum (Fig. 1 and 2). The use of LE-PFA protected against the toxic effects of the free drug, even though the levels of LE-PFA in serum and $t_{1/2}$ were much higher (Fig. 4). Calcium homeostasis is important for the cell to perform normal functions. A perturbation in calcium homeostasis causes the cell to go through several abnormalities that sometimes lead to necrosis or cellular death (22, 24). This metabolic abnormality, which is transient and likely occurs after each dose, may explain, at least in part, the tubular necrosis observed in humans after multiple doses.

PFA has been shown to be nephrotoxic in patients. Up to now, there has been no report on the effects of PFA on calcium, phosphorus, and renal function in animal models. Here we have shown that PFA resulted in a small yet significant increase in the level of creatinine in the sera of mice after 5 days of treatment with 500 mg/kg/day divided into three doses daily. There was no change when a lower PFA dosage of 340 mg/kg/day was used. The BUN level did not change in either case. There is an obvious difference between PFA nephrotoxicity in humans and that in animals (mice in our case). Animals may be more resistant to this drug, or the factors that increase the susceptibility of humans to the nephrotoxic effects of this drug may not exist in animals. In humans, these factors could be the presence of opportunistic infections or other potentially toxic drugs during treatment with PFA.

LE-PFA at up to 35 mg/kg/day did not result in any elevation in the level of either creatinine or BUN in serum for up to 2 weeks of treatment. We have recently shown that unlike free PFA, LE-PFA does not accumulate in the kidney; this may account for its reduced nephrotoxicity (9). Higher dosages could not be tested for that length of time because of technical constraints. Even though the tested dosage of LE-PFA was much smaller than that of the free drug, this dosage produced a C_{max} similar to that of the free drug; it also allowed us to detect its effects on the toxicity parameters measured. Using other animals, some investigators have compared (as we did) the effects of lower concentrations of encapsulated antimicrobial agents with those of higher concentrations of free drugs for similar reasons and/or the stability and/or solubility of the encapsulated drug (6, 27). We have also shown here that the $t_{1/2}$ of LE-PFA is about four times longer than that of free PFA and that the systemic clearance of PFA is five times higher than that of LE-PFA (Table 1).

In conclusion, our results show that PFA increased the levels of creatinine in serum as well as induced the same metabolic changes that are observed in humans, but to a lesser extent. By preventing PFA-induced hypocalcemia and hypophosphatemia, this liposomal formulation might protect against the metabolic changes observed with free PFA. If it is applicable to humans, this liposomal formulation of PFA might be used at lower doses and at longer intervals than is free PFA. Clinical investigations of LE-PFA may be worthwhile.

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