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DISTRIBUTION OF FREE AND LIPOSOMAL CEFOXITIN IN PLASMA AND PERITONEAL FLUID IN A PORCINE INTRA-ABDOMINAL SEPSIS MODEL

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Distribution of Free and Liposomal Cefoxitin in Plasma and Peritoneal Fluid in a Porcine Intra-abdominal Sepsis Model

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The plasma and peritoneal fluid pharmacokinetic parameters obtained after the intravenous administration of free and liposomal cefoxitin were studied in a porcine model of intraabdominal sepsis. No prior assumptions were made to predict the number of compartments pertaining to drug clearance from the administration of either cefoxitin formulation. The experimental data obtained were applied to fit mathematical models of multiexponential drug clearance and the pharmacokientic data were found to best fit a two-compartment open model. Liposomal encapsulation significantly altered the plasma drug distribution pattern resulting in changes in the magnitude of a number of pharmacokinetic parameters examined. The mean post-distributive half-life of liposomal cefoxitin was substantially longer than that of free cefoxitin by at least 3 times. The peritoneal cavity appeared to provide a reservoir for the initial distributive phase of rapid drug clearance from the plasma compartment followed by a less-rapid post-distributive phase. The cumulative drug level, as determined by the area under the concentration curve (AUC) as a function of time, in the plasma of animals treated with liposomal cefoxitin was about 3-4 fold as high as that of animals treated with free cefoxitin. The differences in pharmacokinetic parameters appeared to account for the improved therapeutic efficacy of liposomal cefoxitin in this animal model.

Keywords: Liposome, cefoxitin, sepsis, porcine, drug distribution

INTRODUCTION

Cefoxitin is a semi-synthetic antibiotic from the cephamycin C family, active against Gram-positive and Gram-negative bacteria. It is an important therapeutic agent because of its resistance to destruction by bacterial β-lactamase (Kosmidis et al., 1973; Onishi et al., 1974; Neu, 1974) and cefoxitin is not metabolized appreciably in the body (Brenner, 1982). The high renal clearance of the drug, however, limits the usefulness of this potent antibiotic. The circulating half-life of cefoxitin has been determined to be

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between 30-50 minutes (Brenner, 1982). The relatively short half-life of cefoxitin in the circulation requires the drug to be administered repeatedly, in a multiple-dose regimen to achieve sufficient efficacy for the treatment of post-operative wound infection in patients undergoing appendectomy (Liberman et al, 1995) and open biliary tract surgery (Lapointe et al 1994). In an experimental model of polymicrobial infection after hemorrhagic shock, an increased dosing of cefoxitin was found to be superior to a conventional dose in controlling mixed Gram-negative infection (Livingston and Malangoni, 1993). Cefoxitin has also been demonstrated to be useful in triggering the release of tumor necrosis factor in reducing the fatal outcome in a murine model of mixed *Escherichia coli/Bacillus fragilis* peritonitis (Sawyer et al., 1993). All these studies have demonstrated the usefulness of cefoxitin in treating polymicrobial infections but at the same time, suggest the need for a sustained-release cefoxitin formulation for improved therapeutic results.

Among a wide choice of available drug delivery systems, liposomes, which are phospholipid bilayer vesicles, have been shown to be highly promising, primarily because they are biocompatible, biodegradable and physicochemically flexible to meet specific drug delivery requirements (Shek and Barber, 1986; Shek et al., 1994). Liposomal entrapment has been shown to be useful in preventing premature drug release in the circulation; promoting drug retention time; reducing undesirable systemic side effects; and facilitating site-specific drug delivery (Juliano and Lopez-Berestein, 1985; Gregoriadis and Florence, 1993; Fidler and Kleinerman, 1994; Jones, 1994). Liposomes have been used extensively for the intracellular delivery of therapeutic agents to phagocytes of the reticuloendothelial system (Daemen et al., 1988; Kende et al., 1995) as well as for drug targeting to non-phagocytic cells (Juliano et al., 1987; Suntres et al., 1993; Killion et al., 1994).

In microbial diseases where intracellular microbes are shielded from conventional antimicrobial drugs, the use of liposomes for drug entrapment has been found to enhance therapeutic efficacy by promoting the intracellular uptake of the entrapped drug (Gregoriadis, 1991; Gregoriadis and Florence, 1993). Thus, improved therapeutic efficacy has been demonstrated in using liposomes for the entrapment of antimicrobial drugs in treating a variety of microbial infections (Fountain et al., 1985; Swenson et al., 1990; Chopra et al., 1991; Nightinghale et al., 1993; Wong et al., 1995). In the case of treating experimental intra-abdominal sepsis, liposome-entrapped therapeutic agents have been shown to improve both the bactericidal effect and therapeutic efficacy (Price et al., 1989; Izbicki et al., 1991; Bohnen, 1995; Soltes et al., 1995). Despite the demonstration of improved antibacterial and therapeutic effects of liposome-entrapped cefoxitin, there has been little or no study examining the fate of the liposomal drug after its administration *in vivo* (Kresta et al., 1993).

The present study was undertaken to determine the levels of cefoxitin in the plasma and the peritoneal fluid after its intravenous administration in the free form or as a liposomal preparation in septic pigs. This series of experiments constitutes part of a study examining the bactericidal effect and therapeutic potential of a liposomal cefoxitin formulation in a porcine model of intra-abdominal sepsis.

MATERIALS AND METHODS

Animals

Male Yorkshire Landrace pigs (Albro Farms, Newcastle, Ontario) weighing 18-22 kg were used in this study and they were allowed to acclimatize for at least one week prior to each experiment. Animals were fasted, but allowed water *ad libitum* the night before undergoing surgical procedures. Animals were kept under anaesthesia for the duration of sepsis induction and treatment. Premedication was with intramuscular injections of ketamine (18 mg kg^{-1}), acepromasine maleate (Atrovet) (0.4 mg kg^{-1}) and atropine (0.03 mg kg^{-1}). Endotracheal intubation was performed and the animals were initially allowed to inhale 1% halothane followed by a maintenance dose of pentobarbital of approximately 20 mg kg^{-1} h^{-1}.

The left carotid artery was catheterized for monitoring the mean arterial pressure and also for periodic

blood sampling. A double lumen catheter was inserted into the left external jugular vein for the administration of fluid and anaesthetic. A Swan-Ganz thermodilution catheter was introduced into the pulmonary artery via the right external jugular vein for the measurement of mean pulmonary artery pressure, pulmonary artery wedge pressure, central venous pressure and cardiac output. Details of the animal manipulations have previously been described (Soltes et al., 1995)

The animal studies in this investigation were approved by the Hospital for Sick Children Animal Care Committee and were performed in accordance with the guidelines of the Canadian Council on Animal Care.

Sepsis Induction

Sepsis was induced by an i.p. injection of a fecal inoculum, containing approximately 10^{11} colony forming units of a human clinical isolate of *E. coli* (kindly provided by Dr. O. Rotstein of The Toronto Hospital). Quantitative cultures were performed on all inocula. The inoculated animals were continuously monitored for up to 30 h.

Liposomal Cefoxitin Preparation

Palmitoyloleoylphosphatidylcholine and cholesterol (Avanti Polar Lipids, Inc., Alabaster, AL), at a molar ratio of 55:45, were dissolved in a minimal volume of chloroform. The lipids were dried into a thin film under a stream of helium and residual solvent was removed *in vacuo* overnight. The lipid film was rehydrated, at room temperature, with sterile saline containing cefoxitin at an initial drug to lipid ratio of 710 mg cefoxitin/mmol total lipid in a final volume of 5.5 mL. The liposomes formed were vortexed periodically for 30 min and then extruded repeatedly (10 times) through an extruder (Lipex Biomolecules Inc., Vancouver, BC) fitted with polycarbonate membrane filters (Nuclepore Corp., Pleasanton, CA) of 100-nm pore-size. The resulting extruded liposomal cefoxitin preparation, adjusted to an appropriate concentration required by the experimental protocol, was used for intravenous administration in animals. Particle size analysis of the extruded vesicles with a Coulter N4SD sub-micron particle analyser revealed a unimodal distribution with a mean vesicle diameter of 103 ± 30 nm (SD).

Drug Administration

Six hours following the intraperitoneal injection of a fecal inoculum of *E. coli*, the animals were given an intravenous injection of either free or liposomal cefoxitin, at a dose of 30 mg/kg body weight in a total volume of 30 mL. Each drug preparation was administered over a 10-min period and an identical drug administration was repeated 6 hours later.

Biological Specimen Collection

Blood and peritoneal fluid were collected in EDTA tubes from experimental animals. During the first 30-min period after drug administration, blood samples were withdrawn every 5 min; in the second 30-min period, every 10 min; in the next 60-min period, every 15 min; in the following 2-h period, every 30 min; and hourly thereafter. The same blood collection protocol was used following the second injection of antibiotic. Plasma was separated from each blood sample and kept frozen at −70°C until use.

Cefoxitin Measurement

Sample preparation

A fresh solution of cefoxitin (Sigma Chemical, St. Louis, MO) was prepared for each determination and precautions were taken to minimize or prevent the drug solution from exposure to strong light. An internal standard of cefuroxime (100 ng/10 μL saline) was added to 100 μL of pig plasma; mixed with 210 μL of methanol and 100 μL of chloroform; and vortexed and centrifuged at 2,500 *g* for 5 min. The supernatant was then mixed with 100 μL chloroform followed by vortexing and centrifugation under the same conditions as before. After the removal of the organic layer, the water/methanol layer was dried

almost to completion under a stream of nitrogen at 40°C. The dried samples were dissolved in 100 μL of 1% formic acid and filtered through 4-μm and 0.47-μm Nylon syringe filters. Aliquots of the samples (10-20 μL) were then injected into the liquid chromatography/mass spectrometry (LC/MS) system for analysis.

LC/MS analysis

The cefoxitin assay was performed using the Finnigan TSQ-700 MS/MS System equipped with a Finnigan APCI/ESI LC/MS interface and Waters 600 HPLC with a WHISP autosampler. The conditions for running the HPLC were as follows: 12.5 cm × 4.7 mm Whatman 5-μm PartiSphere C-18 reverse phase column fitted with a pre-column filter; mobile phase methanol:1% formic acid (40:60); and a flow rate of 0.3 mL/min. The retention time of cefoxitin was 16 min. The conditions for operating the mass spectrometer were as follows: MS electrospray source spray set at 1,500 V producing a current about 1.5mA; capillary heater set at 240°C, sheath gas nitrogen pressure set at 40 psi; and the skimmer pump pressure at 800 mtorr. The mass spectrometer was used in a negative ion mode using one of the following 2 methods: 1) the first quadrupole sector was scanned from 422-427 amu in 5 sec and areas under masses 423 m/z (cefuroxime) and 426 m/z (cefoxitin) were monitored or 2) daughter ions 318 m/z and 336 m/z of cefuroxime parent 423 m/z and, daughter ions 333 m/z of cefoxitin parent 426 m/z were monitored by selective reaction monitoring (SRM). The manifold temperature was set at 80°C and the electron multiplier, equipped with 5 kV dynodes, was set at 1,500 V. The calibration curves were prepared by spiking 100 μL of blank pig plasma with cefoxitin (0.01, 0.1, 1, 5, and 10 μg/mL) and 100 ng of cefuroxime as an internal standard. The area ratios of cefoxitin/cefuroxime were plotted against a concentration of cefoxitin. The coefficient of variation for within assay was <5% (n = 10) and between assay, <10% (n = 5).

Calculations of Pharmacokinetic Parameters

It is customary to express the kinetics of drug clearance as an exponential decay function. Bi-exponential decays imply a second compartment (Klaassen, 1980). No assumptions were made about the number of compartments that pertain to cefoxitin clearance; rather, the decision on whether one *vs* two compartments apply was determined through statistical analysis. We began by applying the single exponential one-compartment model for drug clearance:

$$conc = A \bullet \exp(-a \bullet t)$$

where A is the intercept plasma concentration of cefoxitin (i.e., at time zero) and a is the corresponding elimination rate constant. The above expression was fitted to the drug clearance data through non-linear regression (Dixon, 1983). A measure of the goodness of fit between the predicted and observed concentrations is the sum of squared residuals (SSR). Adding a second compartment, i.e.,

$$conc = A \bullet \exp(-a \bullet t) + B \bullet \exp(-b \bullet t)$$

will improve the fit and reduce the SSR. However, the improvement is significant only if the ratio of SSRs exceeds a threshold value based on Fisher's F-test (Mekajavic and Morrison, 1986). With 15 observations per trial and 2 estimated parameters per compartment, the significant F-value at the 95% level is 4.26 and, therefore, improvement in fit is significant if the ratio of SSRs exceeds 1.95. No attempt was made to fit the data to a 3-compartment model since the data are insufficient to support additional parameter estimations.

The pharmacokinetic parameters derived from the above fits of data are half-life ($t_{1/2}$), area under the plasma drug concentration curve (AUC), total systemic clearance (Cl), and the volume of distribution (Vd). The half-time is the reciprocal of the elimination rate constant (a or b) multiplied by the natural logarithim of 2. AUC is calculated according to the integrated value (from time zero to infinity) of the fitted concentration profile, i.e.,

$$AUC = A \bullet a^{-1} + B \bullet b^{-1}$$

which accounts for the entire clearance of the drug. Cl is the dose (30 mg•kg^{-1}) divided by AUC. Finally, V_d is determined by the ratio $Cl \bullet k_{kel}^{-1}$ where k_{el} is the 2-compartment elimination rate constant given by:

$$Kel = (a \bullet B + b \bullet A) \bullet (A + B)^{-1}$$

As pointed out by Klaassen (1980), k_{el}^{-1} does not represent the time constant but is often referred to as such.

Data Analysis

The above 1- and 2-compartment models were applied to the clearance data of each pig separately. It was found that the additional compartment improved the fit significantly in 64% of the total pig trials. However, for consistency when grouping the results of all pigs, the 2-compartment model parameter estimations were used. The pharmocokinetic parameters reported under Results are the mean of the pigs' values with dose as the grouping factor.

In the case of the pigs injected with liposome cefoxitin, the drug was still present at the start of the second dose (i.e., 6 h after the first dose). To account for this residual (or 'spillover') cefoxitin, its value was predicted from the clearance model derived from the first dose and subsequently subtracted from the measured value of the second dose; i.e.,

$$\begin{aligned} &\text{corrected } conc_2 \\ &= \text{measured } conc_2 - A_1 \bullet \exp\{-a_1 \bullet (t + 6)\} \\ &\quad - B_1 \bullet \exp\{-B_1 \bullet (t + 6)\} \end{aligned}$$

where the subscripts 1 and 2 of *conc* pertain to the first and second doses, respectively. All reported pharmacokinetic parameters were derived from the corrected cefoxitin concentrations, where applicable.

Analysis of Peritoneal Fluid Concentration of Cefoxitin

The appearance of cefoxitin in the peritoneal fluid exhibits an initial rapid increase in concentration followed by a slower exponential decay. An appropriate fitting function to model this behaviour is described by:

$$conc = A \bullet \{\exp(-a \bullet t) - \exp(-c \bullet t)\}$$

where the uptake rate constant c has a larger value than the elimination rate constant a. The data are insufficient to consider more sophisticated models.

Pharmacokinetic parameters can be derived from the above expression; however, in a slightly different manner than with the plasma cefoxitin clearance model. The half-life should be solely based on the elimination rate constant, hence $k_{el}=a.\bullet AUC$ is the integral of the above expression in:

$$AUC = A \bullet (a^{-1} - c^{-1})$$

and the remaining parameters were calculated as before.

RESULTS

Plasma Cefoxitin Concentration and Pharmacokinetic Parameters

Plasma cefoxitin concentrations resulting from the two separate, single intravenous injections (6 h apart) of liposomal cefoxitin and free cefoxitin in septic pigs are shown in Figure 1. The fitted profiles are based on the mean of the best-fit parameters (Table I) of each pig in their dose grouping. The means of the pharmacokinetic parameters derived from each pig's fitted model values are listed in Table II. Note that these values are derived from the values of individual pigs, rather than from the mean values listed in Table I.

The cumulative drug concentration as a function of time, as defined by the AUC, was found to be significantly higher in animals treated with liposomal cefoxitin than that treated with free cefoxitin (Figure 1, Table II). Based on a comparison of the AUCs between treatments (1-factor ANOVA between groups), liposomal encapsulation provided a significantly higher amount of drug in the circulation for the 6-h period each after the first and second administered drug dose. The AUC of the liposomal drug treatment was about 3 times and 2 times larger than that of the free drug, after the administration of the first and second drug-dose, respectively.

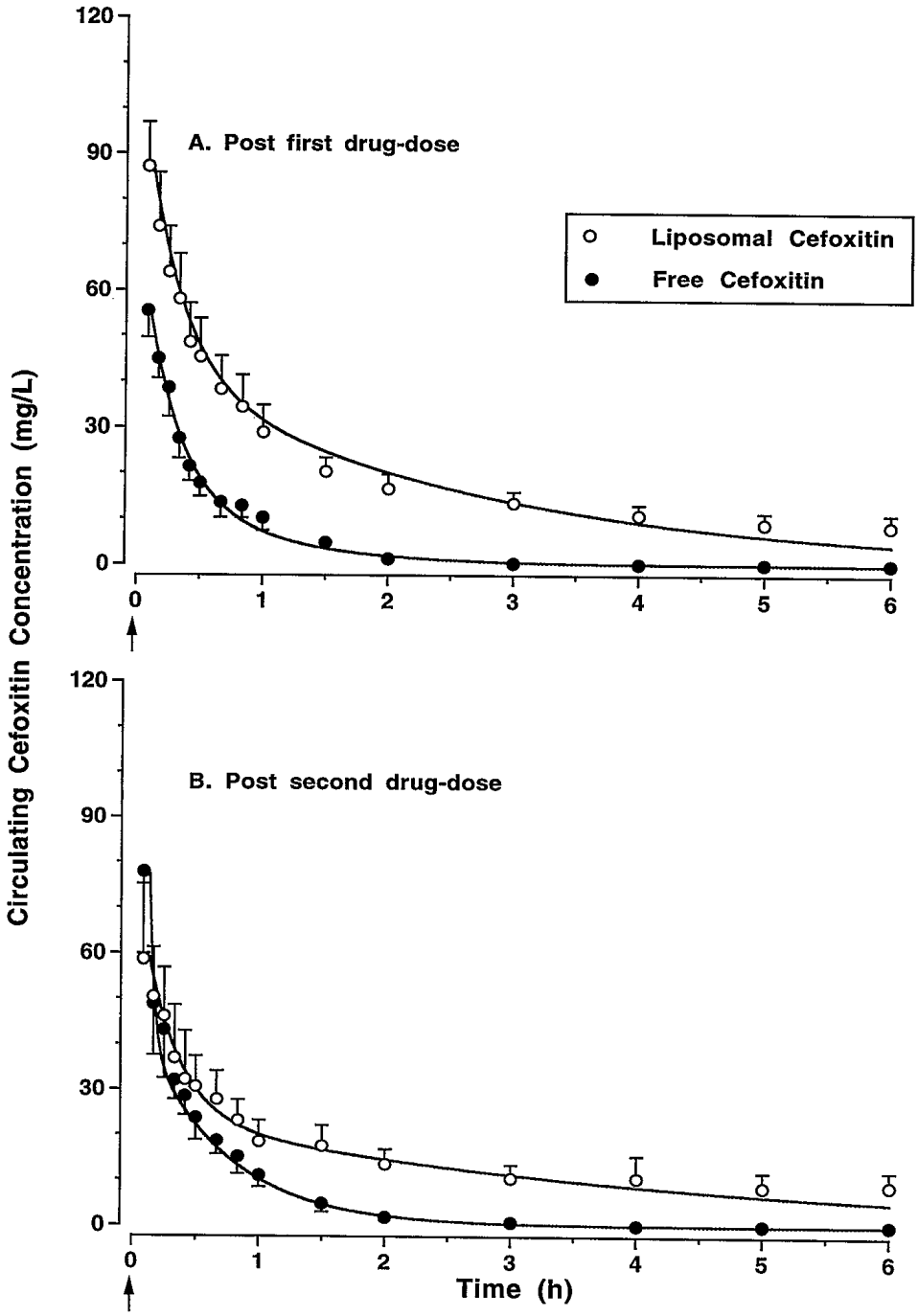

FIGURE 1 Plasma cefoxitin concentrations in septic pigs. Animals were given an intravenous injection of free cefoxitin or liposomal cefoxitin (30 mg/kg body weight) 6 h after a fecal inoculum containing 10^{11} CFU *E. coli* as described in Materials and Methods; serial blood samples were collected at the indicated time-points. Each arrow on the time-axis denotes the injection of a cefoxitin preparation and each data point represents the mean plasma cefoxitin concentration ± SEM (liposomal-cefoxitin-treated animals, n = 6; free-cefoxitin-treated animals, n = 5).

TABLE I Mean of the best-fit parameters in septic animals

Best-fit Parameters	Post first drug-dose		Post second drug-dose	
	Free Cefoxitin	Liposomal Cefoxitin	Free Cefoxitin	Liposomal Cefoxitin
A [mg.L^{-1}]	54.84 ± 12.41	70.25 ± 7.76	1337 ± 978	59.25 ± 14.01
a [h^{-1}]	4.05 ± 0.90	3.55 ± 1.04	26.37 ± 11.20	3.84 ± 1.24
B [mg.L^{-1}]	21.88 ± 5.02	34.98 ± 5.60	48.76 ± 11.52	20.95 ± 1.70
b [h^{-1}]	1.26 ± 0.19	0.39 ± 0.11	1.57 ± 0.21	0.27 ± 0.07

A and B, intercepts of plasma cefoxitin concentration (at time zero) of compartment A and compartment B, respectively; a and b, the corresponding elimination rate constant. Mean ± SEM derived from animals administered with free cefoxitin (n = 5) or liposomal cefoxitin (n = 6)

TABLE II Mean pharmacokinetic parameters of free and liposomal cefoxitin in plasma of septic pigs

Pharmacokinetic Parameters	Post first drug-dose		Post second drug-dose	
	Free Cefoxitin	Liposomal Cefoxitin	Free Cefoxitin	Liposomal Cefoxitin
$t_{1/2\,\alpha}$ [h]	0.21 ± 0.04	0.32 ± 0.12	0.16 ± 0.11	0.39 ± 0.21
$t_{1/2\,\beta}$ [h]	0.59 ± 0.07	2.64 ± 0.63*	0.48 ± 0.07	4.48 ± 1.79*
AUC [mg.min.mL^{-1}]	1.98 ± 0.21	8.39 ± 1.06*	3.75 ± 0.99	10.22 ± 4.42*
Kel [h^{-1}]	2.40 ± 0.37	0.83 ± 0.13*	14.30 ± 7.91	0.77 ± 0.25*
Cl [mL.min^{-1}.kg^{-1}]	15.93 ± 1.78	3.91 ± 0.56*	10.42 ± 2.48	5.10 ± 1.27*
Vd [L.kg^{-1}]	0.42 ± 0.05	0.29 ± 0.02*	0.26 ± 0.17	0.44 ± 0.07*

Mean pharmacokinetic parameters ± SEM were calculated by applying a two-compartment model.
Abbreviations: $t_{1/2}$, half-life; AUC, area under the concentration curve; kel, elimination rate constant; Cl, total systemic clearance; Vd, volume of distribution.
*Statistically significant at $p<0.05$ comparing the mean parameter of liposomal-cefoxitin-treated animals (n = 6) with that of free-cefoxitin-treated animals (n = 5) after the corresponding drug-dose

The administration of liposomal cefoxitin altered the pharmacokinetics of the drug (Table II). The half-life parameter of the first 'fast' model compartment, however, is not significant between the two treatments. This did not seriously impact overall drug clearance, since the second compartment dominates in terms of its contribution to the pharmacokinetic parameters. According to the mean parameter values listed in Table I, the integrated drug concentration of the second compartment (i.e., $B \bullet b^{-1}$) accounts for 81.9 and 83.4% of the total AUC for the first and second doses, respectively. The remaining parameters in Table II are significantly different between the two groups of animals treated with free or liposomal cefoxitin. In both cases after the first and second dose of drug, $t_{1/2\,\beta}$ of the post-distributive phase was substantially longer in liposomal-cefoxitin-treated than in free-cefoxitin-treated animals (about 3.5 times longer after the first dose and 8 times longer after the second dose), resulting in a corresponding increase in AUC and a decrease in the elimination constant. The total systemic clearance and volume of distribution of the liposomal drug were also significantly different from those of the free drug.

Peritoneal Fluid Concentration of Cefoxitin

Peritoneal cefoxitin concentrations resulting from the two separate, single intravenous injections of liposomal cefoxitin and free cefoxitin in septic pigs are shown in Figure 2. The fitted profiles are based on the fit of the mean of the pigs' cefoxitin concentration in their respective dose grouping. This departure from using the mean of the best-fit parameters (see Table III) to generate the profiles, as done previously, is due to the resultant poor goodness of fit owing to the large variability in pig responses.

The partitioning of the intravenous drug into the peritoneum appeared to show a pattern of attaining higher peritoneal drug levels among animals treated

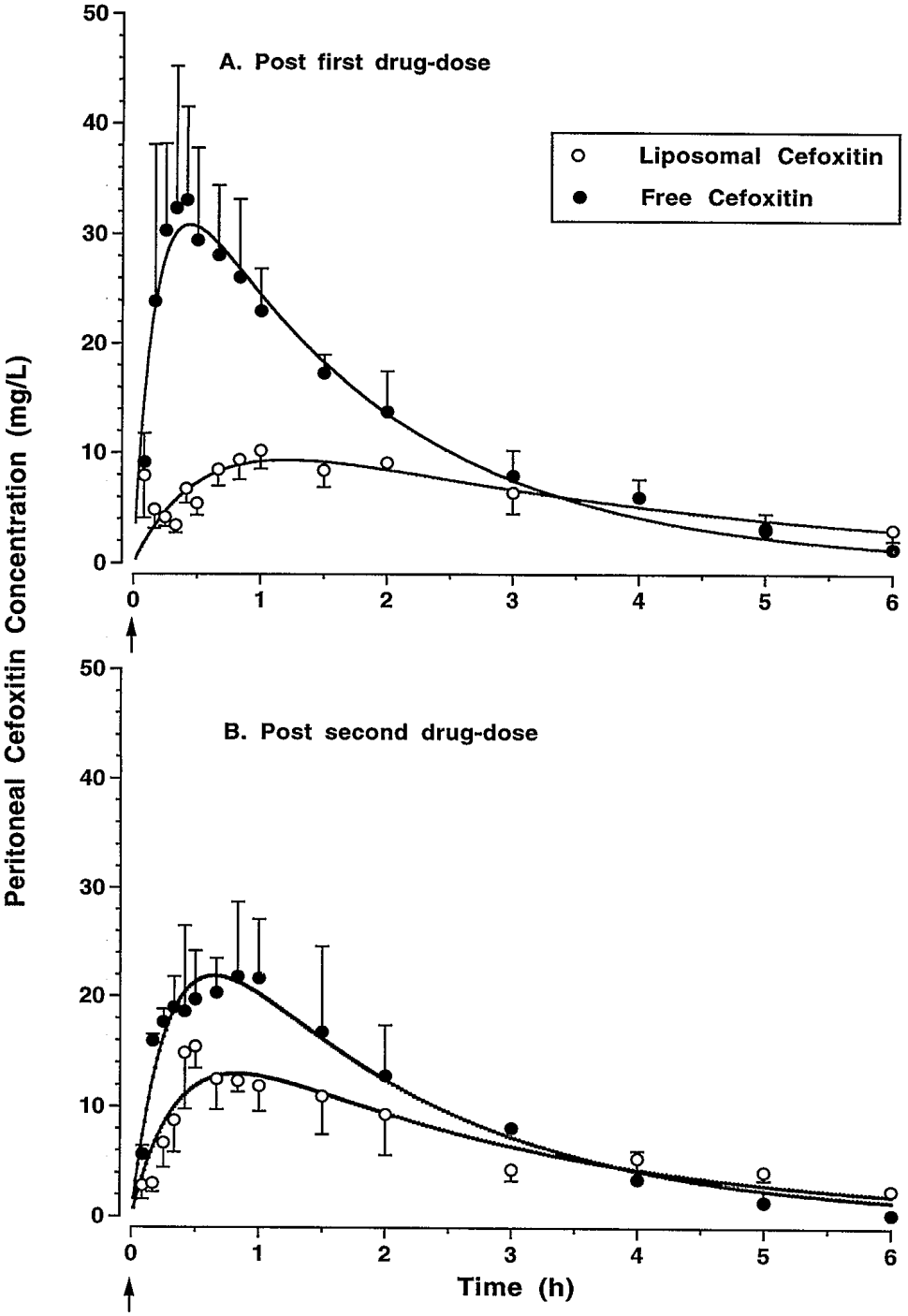

FIGURE 2 Peritoneal cefoxitin concentrations in septic pigs. Animals were given an intravenous injection of free cefoxitin or liposomal cefoxitin (30 mg/kg body weight) 6 h after an intraperitoneal injection of a fecal inoculum containing 10^{11} CFU *E. coli* as described in Materials and Methods; peritoneal fluid samples were collected at the indicated time-points. Each arrow on the time-axis denotes the injection of a cefoxitin preparation and each data point represents the mean cefoxitin concentration ± SEM of 3 animals in each treatment group.

TABLE III Mean of the best-fit and pharmacokinetic parameters of free and liposomal cefoxitin in peritoneal fluid of septic pigs

Parameters	Post first drug-dose		Post second drug-dose	
	Free Cefoxitin	Liposomal Cefoxitin	Free Cefoxitin	Liposomal Cefoxitin
Best-fit parameters				
A [mg.mL^{-1}]	46.61	35.62	100.50	32.89
c [h^{-1}]	5.39	1.87	4.61	2.27
Pharmacokinetic parameters				
AUC [mg.min.mL^{-1}]	3.98±0.92	3.57±1.06	3.45±0.39	2.55±0.42
kel [h^{-1}]	0.62±0.01	0.31±0.11	0.68±0.31	0.54±0.05
Cl [mL.min^{-1}.kg^{-1}]	7.97±1.84	9.81±2.38	8.81±0.99	12.48±2.19
Vd [L.kg^{-1}]	0.77±0.19	2.32±0.63	1.05±0.57	1.41±0.24

Abbreviations: AUC, area under the concentration curve; kel, elimination rate constant; Cl, clearance; Vd, volume of distribution; A, intercept of cefoxitin concentration of compartment A; c, uptake rate constant. Mean pharmacokinetic parameter ± SEM of 3 animals.

with free cefoxitin than with liposomal cefoxitin (Figure 2). The uptake rate constant (c) of the drug also shows a trend of faster uptake of free drug than liposomal drug in the peritoneal cavity (Table III). The AUC and other pharmacokinetic parameters, however, are not significantly different between the two treatments. The small number of pigs, coupled with their large individual variability, explains the lack of any statistical difference found in the pharmacokinetic parameters, either within groups or between groups.

DISCUSSION

Cefoxitin is a β-lactam antibiotic which is active against different strains of Gram-negative bacteria, including *E. coli* used in the fecal inoculum for the induction of sepsis in this study. Using the same porcine intraabdominal sepsis model, we have previously shown that the bactericidal efficacy of liposomal cefoxitin surpasses that of the free drug (Soltes et al., 1995). In this study, we took advantage of our large animal model to collect blood and peritoneal fluid samples at multiple time-points from the same animal, in order to gain a better understanding of the difference in pharmacokinetics between the liposomal and free cefoxitin treatments.

The plasma clearance pattern of intravenously administered cefoxitin depends on the formulation of the drug. The pharmacokinetics of cefoxitin were significantly altered when the drug was encapsulated in liposomes compared to that of free cefoxitin in the aqueous form. Our results demonstrated that liposomal encapsulation appeared to prevent premature drug release and therefore promote the recirculation time of the drug in the vascular system.

Cefoxitin is not metabolized appreciably in the body and less than 2% is eliminated by metabolism and biliary clearance (McEvoy et al., 1985). The drug is excreted by the kidney and has a terminal half-life of 30-50 min in humans (Brenner, 1982). While the half-life of free cefoxitin is relatively short in the α (distributive) phase in our porcine model ($t_{1/2\,\alpha}$ = 10-13 min), the mean half-life of free cefoxitin in the β (post-distributive) phase ($t_{1/2\,\beta}$ = 29-35 min) is comparable to that in man. The encapsulation of cefoxitin in liposomes, however, substantially increased $t_{1/2\,\beta}$ by about 3.5 times after the first dose and about 9 times after the second dose. This magnitude of liposome-mediated increase in half-life is very similar to that observed in mice given an i.v. injection of liposomal gentamycin versus free gentamycin (Swenson et al., 1990). While there was no significant difference in the half-life of free cefoxitin between the two treatments, the second administration of liposomal cefoxitin demonstrated a longer half-life (4.48 h) than the first injection (2.64 h), although the difference was not statistically significant.

Although not essential in this study, the experiment was repeated with non-septic pigs to observe whether the characteristics of drug clearance were affected by the health of the animal. No significant differences

were found between the pharmacokinetic parameters of the free and liposomal cefoxitin. However, this may be attributable to the small number of non-septic pigs used (3 in each group). When compared to the pharmacokinetic parameters of the septic pigs, values obtained from non-septic animals are quite similar (data not shown). Judging from the strength of the similarities between these values and those listed in Table II for the septic pigs, where almost twice as many animals were used and significant differences in drug clearance were found, we propose that the longer retention of liposomal cefoxitin is independent of the pig's condition.

Based on the mathematical model fitting of the experimental data in this study, it is clear that the pharmacokinetic parameters best fit a two-compartment model with biexponential drug removal from the plasma. Since it is known that cefoxitin widely distributes into body fluids and tissues, our observation of an at least two-compartment system with free or liposomal cefoxitin is not surprising. Our results also provide evidence to suggest that the peritoneal cavity may serve as part of the second compartment for the rapid escape of the circulating drug. This concept is well supported by the fact that the fast increase in peritoneal cefoxitin concentration appeared to correspond to an equally fast decrease in drug clearance from the plasma compartment within the first 30 min of drug administration. This apparent two-compartment equilibrium was also observed in a septic rodent model, where free and liposomal cefoxitin were injected intraperitoneally and a reversed distribution occurred with the drug redistributing from the peritoneal cavity to the circulation (Kresta et al., 1993). The prolonged half-life of the liposomal formulation is likely to provide a sustained release action, possibly promoting its bioavailability at the site of action.

The i.v. adminstration of free cefoxitin declined to a level in the plasma below its minimal inhibitory concentration (MIC) of 6 μg/mL for this strain of *E. coli* 2 h after injection. In contrast, the plasma drug level in animals treated with liposomal cefoxitin was maintained far above the MIC for at least 6 h after the first and second administered dose in each case. The plasma cefoxitin, at a quantified concentration at or above the MIC, was found to be pharmacologically active in an *in vitro* biological assay (data not shown), demonstrating that the circulating drug was potentially available for further antimicrobial action.

In this large animal model, it is impractical to obtain tissue samples from various organs, at the corresponding timepoints of blood sampling, for drug distribution analysis. However, it is conceivable that the liposomal formulation, with a longer recirculation time in the vasculature, should be able to reperfuse various organs with a higher drug dose than the conventional drug formulation. Indeed, using confocal laser scan microscopy and microfluorometry to determine tissue distribution and to quantitate drug uptake, Vaage et al. (1994) also demonstrated a decrease in systemic elimination and an increase in drug delivery of intravenously administered liposomal doxorubicin to the liver than free doxorubicin in a rodent tumor model. The encapsulation of the aminoglycoside antibiotic amikacin and its administration via the intravenous route has been shown to be effective against *Mycobacterium avium-intracellulare* complex in the livers, kidneys, and spleens of mice, while the free antibiotic at the same dose was ineffective (Duzgunes et al., 1988). We previously reported that liposome-encapsulated cefoxitin attained a substantial increase (30-fold) of drug level in the liver compared to the administration of free cefoxitin in a rodent model of intraabdominal sepsis (Kresta et al., 1993). We have also reported that i.v. liposomal cefoxitin confers a substantially improved bactericidal effect than free cefoxitin in the livers and lungs of infected animals in a porcine sepsis model identical to the one adopted in this study (Soltes et al., 1995). Therefore, it is quite possible that the enhanced hepatic and pulmonary bacterial clearance can be partly explained by the significantly higher drug levels perfusing the infected organs. Since it has been well established that liposomal drugs are preferentially taken up by reticuloendothelial cells via phagocytosis (Scherphof et al., 1983; Duzgunes, 1991; Bohnen, 1995), the decreased hepatic *E. coli* CFU counts in the treated septic pigs may also be

attributable to the intracellular delivery of liposome-encapsulated cefoxitin to phagocytic (Kuppfer) cells of the liver.

In support of the generally improved biological efficacy of liposomal antimicrobial drugs, the results of the present study enable us to consider the intricate relationship of drug redistribution from the central plasma compartment to a peripheral site, for example, the peritoneal cavity. Such information is vital in the design of drug formulation and drug dosing for effective site-specific therapy (Bergogne-Berezin, 1996). In conclusion, our results support the notion that liposome encapsulation can prolong the circulating half life of an encapsulated drug, there by possibly promoting drug targeting and improving drug efficacy.

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