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Enhanced Oral Bioavailability and Intestinal Lymphatic Transport of a Hydrophilic Drug Using Liposomes

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ABSTRACT A liposome system was evaluated for oral delivery of a poorly bioavailable hydrophilic drug. The system was prepared from proliposome, which consisted of negatively charged phosphatidylcholine, whereas cefotaxime was chosen as the model drug. An in vivo study was carried out on nine rats according to a three-way crossover design to compare the oral bioavailability of cefotaxime from the liposomal formulation with that of an aqueous drug solution and a physical mixture of cefotaxime with blank liposomes. The results indicated that the extent of bioavailability of cefotaxime was increased approximately 2.7 and 2.3 times compared with that of the aqueous solution and the physical mixture, respectively. In a separate study, simultaneous determination of cefotaxime in intestinal lymph (collected from the mesenteric lymph duct) and in plasma (collected from the tail vein) revealed that its concentration was consistently higher in the lymph than in the plasma when administered via the liposomal formulation, whereas the reverse was observed with the aqueous solution. Thus, the results indicated that the liposomes system has the potential of increasing the oral bioavailability of poorly bioavailable hydrophilic drugs and also promote their lymphatic transport in the intestinal lymph.

KEYWORDS Liposomes, Oral administration, Hydrophilic drug, Cefotaxime, Lymphatic transport

INTRODUCTION

The oral route is by far the easiest and most convenient way of drug administration especially when repeated or routine dosing is necessary (Chen and Langer, 1998). However, many drugs show no significant or poor bioavailability when administered orally with conventional dosage forms due to chemical instability or poor absorption from the gastrointestinal tract (Weiner and Chiang, 1988).

As a result, new formulations consisting of novel delivery vehicles including polymeric particles, liposomes, and bioadhesive particulates (Takeuchi et al.,

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1996; Sharma and Sharma, 1997; Chen and Langer, 1998) have been investigated in an attempt to circumvent these problems. Among the novel drug delivery systems, the use of liposomes in the delivery of therapeutic agents, such as antimicrobial drugs, anticancer drugs, steroids, peptides, enzymes and hormones, has been much investigated (Gregoriadis and Florence, 1993; Sharma and Sharma, 1997). Several studies showed that liposomes facilitate the gastrointestinal absorption of drugs by protecting labile compounds from degradation or by increasing the rate and/or extent of uptake of drugs (Dapergolas et al., 1976; Weiner and Chiang, 1988). However, most of the studies involved entrapment of lipophilic drugs (Nagata et al., 1984; Yotsuyanagi et al., 1988; Al-Meshal et al., 1998; Bayomi et al., 1998). Studies on hydrophilic drugs using oral liposomal delivery are somewhat limited.

Orally administered drugs can gain access to the systemic circulation via portal blood and the intestinal lymphatics. Drug transport via the intestinal lymphatic system has been shown to contribute to the absorption of a number of highly lipophilic compounds such as lipid-soluble vitamins (Fukui et al., 1989), DDT (Noguchi et al., 1985), halofantrine (Porter et al., 1996), CI-976 (Hauss et al., 1998), and MK-386 (Kwei et al., 1998) when administered orally. Although portal blood represents the major absorption pathway with its high capacity to transport both hydrophilic and hydrophobic drugs, the intestinal lymphatic system offers the advantage of bypassing the liver and hence increases the systemic bioavailability of drugs with high first-pass metabolism (O'Driscoll, 1992).

Here, we described the development of a liposome delivery system with the potential of enhancing the oral bioavailability of poorly absorbed hydrophilic drugs. In addition, the role of liposomes as a lipophilic carrier in increasing lymphatic transport of hydrophilic drugs was investigated. Cefotaxime was used as a model drug. It is water soluble, is not stable in acidic conditions, and has negligible bioavailability when given orally as an aqueous solution. Proliposome was used to prepare the liposomal formulation because of the ease of preparation.

MATERIALS AND METHODS

Materials

Pro-lipo duo® (Lucas Meyer, France), a specially formulated proliposome containing 50% of negatively charged unsaturated soybean phosphatidylcholine suspended in hydrophilic medium consisted of glycerol and ethanol, was used. Cefotaxime sodium was obtained from Orchid Chemicals and Pharmaceuticals (Chennai, India). Triton X-100 (t-Octylphenoxypolyethoxyethanol) was purchased from Sigma (St. Louis, MO, USA). Sagatal® (Pentobarbitone sodium 60 mg/mL) was obtained from Rhône Mérieux (Harlow, Essex, UK). Polyethylene (PE) tubing (0.5 mm i.d., 0.8 mm o.d.) used for cannulation of mesenteric lymph duct was obtained from Dural Plastics and Engineering (Auburn, NSW Australia). PE 120 and PE 50 tubings were obtained from Watsons (United Kingdom). Disodium ethylenediaminetetracetate (EDTA) was obtained from Merck BDH (Poole, UK). Cyanoacrylate glue was obtained from Loctite (Dublin, Ireland). All needles and syringes were obtained from Becton Dickinson (Singapore). High-intensity fiber-optic illuminator was purchased from Cole Parmer (Vernon Hills, IL, USA) while stereo microscope was obtained from Kyowa Optical (Tokyo, Japan). All surgical tools were obtained from Aesculab (Tuttlingen, Germany). All solvents and chemicals used were of AR (analytical reagent) or HPLC (high-performance liquid chromatography) grade.

Preparations

Cefotaxime aqueous solution at a concentration of 5 mg/mL was prepared by dissolving cefotaxime in distilled water. Pro-lipo duo® was used as received. This proliposome mixture was converted to concentrated cefotaxime-loaded liposome preparation by dropwise addition of cefotaxime aqueous solution (20 mg/mL) with moderate stirring for 30 min at room temperature. The concentrated liposomal preparation was then gradually diluted to a predetermined volume with distilled water to achieve a final concentration of 5 mg/mL cefotaxime. The ratio of Pro-lipo duo®:drug solution:distilled water was 1:2:5 w/w/w, as recommended by the manufacturer. As for the physical mixture, blank liposomes (entrapping distilled water in its aqueous phase) were first prepared by dropwise addition of distilled water and then followed by dilution with cefotaxime aqueous solution (20 mg/mL). The physical mixture has the same composition as the drug-loaded liposomal preparation above in terms of the amount of drug, proliposomes, and distilled water used. Both cefotaxime-loaded liposomes and physical mixture of cefotaxime with blank liposomes were prepared extemporaneously and were used as prepared.

Particle Size Analysis

The particle size of the blank liposomes, cefotaxime-loaded liposomes, and the blank liposomes in the physical mixtures were estimated by photon correlation spectroscopy (Zetasizer 1000 HS, Malvern, Worcestershire, UK). The distilled water used for dispersing the liposomes was filtered through 0.22 μm, white GSWP, 47 mm Millipore filter (Bedford, MA, USA). A total of three batches for each formulation were prepared, and three measurements were taken on two separate samples from each batch. The Z average diameter (ZAve), which is a natural intensity weighted mean and polydispersity index were used as parameters of mean particle size and size distribution, respectively.

An analysis of variance procedure (one-way ANOVA) appropriate for a parallel group design was used to compare the ZAve values of blank liposomes, cefotaxime–loaded liposomes, and the physical mixtures. This was followed by Tukey's test if a statistically significant difference was detected. A statistically significant difference was considered at $P < 0.05$.

Entrapment Efficiency Determination

Entrapment efficiency is defined as the percent fraction of the total input drug encapsulated in the liposomes, at a particular phospholipid concentration (Kulkarni et al., 1995). It was expressed as mg cefotaxime entrapped per 100 mg phospholipid. The percentage of drug entrapped in the liposomes was calculated by using the following formula:

$$\% \text{ entrapped} = \frac{[\text{total cefotaxime}] - [\text{free cefotaxime}]}{[\text{total cefotaxime}]}$$

Separation of cefotaxime

The cefotaxime-containing liposomes were separated from the free (unentrapped) cefotaxime by ultracentrifugation at 50,000 rpm and 20°C for 2 h (Beckman Optima L-80, USA). Duplicate samples were used. The supernatant consisted of free cefotaxime was collected and kept frozen at –20°C until analysis.

Assay of the Free and Total Cefotaxime

Triton X-100 1% (w/w) was added to liposome suspension at 1:1 ratio (v/v) to destroy the phospholipid bilayer structures, freeing the drug. The mixture was vortexed for 30 sec (Barnstead/Thermolyne, Dubuque, IA, USA) followed by centrifugation for 15 min at 12,800 rpm (Eppendorf, Hamburg, Germany). Duplicate samples were used. Clear supernatant was transferred to a new microcentrifuge tube and kept frozen at –20°C until analysis. The concentrations of cefotaxime were analyzed by using an HPLC method reported earlier (Ling et al., 2003).

Animal Experiments

Two separate studies were carried out by using rats. The first study was aimed to investigate the potential of the liposomal preparation in enhancing the oral bioavailability of a poorly bioavailable hydrophilic drug, whereas the second experiment was to evaluate the effect of liposomes on the lymphatic transport of the hydrophilic drug after absorption. All experimental and surgical procedures were reviewed and approved by the Ethics Committee on Animal Studies, University of Science Malaysia.

Study Protocols

Study I

The experiment was carried out by using 9 adult male, Sprague-Dawley rats weighing 193–278 g (mean = 233 g; SD = 18 g), according to a 3-period, 3-sequence crossover design, with a 1-week washout period between phases. The rats were randomly divided into 3 groups of 3 rats each. The rats in different groups were administered with different preparations according to the sequence shown in Table 1. The animals were fasted for 12 h before drug administration and also during the sampling period but were allowed free access to water throughout the experiment; 3.2 mL each of cefotaxime aqueous solution, liposomal formulation, and physical mixture (each containing 16 mg of cefotaxime) was administered intragastrically by using stainless steel animal feeding needles followed by an additional 1.0 mL of water. The animals were subsequently placed in restraining cages, and their tails were clipped (Patton & Gilford 1981). Blood samples (approximately 0.5 mL) were collected from the tail vein into heparinized tubes at 0 (before administration), 0.5, 1, 1.5, 2, 3, 5, 7, and 9 h postadministration. The blood samples were then centrifuged for 20 min at 12,800 rpm. About 0.2–0.3-mL aliquot of plasma

TABLE 1 Sequence of Administration of Different Preparations

Group	Sequence of administration		
	1st week	2nd week	3rd week
Group I	Drug solution	Liposomal formulation	Physical mixture
Group II	Liposomal formulation	Physical mixture	Drug solution
Group III	Physical mixture	Drug solution	Liposomal formulation

obtained from each blood sample was transferred into a new microcentrifuge tube. All plasma samples were stored frozen at –20°C until analysis.

Study II

The experiment was carried out by using 10 adult male, Sprague-Dawley rats weighing 301–400 g (mean = 361 g; SD = 33 g). The rats were randomly divided into two groups of five rats each with one group receiving cefotaxime aqueous solution, whereas the other group received the liposomal formulation. Nonfasted rats were used for better visualization of the mesenteric lymph duct. The rats were allowed controlled amount of normal pellet diet a day before the surgery.

Surgical anesthesia was initiated in the rats by intraperitoneal (i.p.) injection of 60 mg/kg of Sagatal® (Pentobarbitone sodium 60 mg/mL) using 1 mL syringe with 27G1/2 needle. Additional i.p. doses of 15–20 mg/kg of the anesthetic agent were administered as and when required (every 1–2 h) to maintain anesthesia. A 2-cm piece of PE 120 tubing was inserted into the trachea, and the respiratory secretion was cleared by regularly sucking it out with a syringe with 23G11/4 TW needle attached to a 5-cm piece of PE 50 tubing. Mesenteric lymph duct was cannulated for the collection of intestinal lymph, whereas systemic blood samples were collected from the tail vein. Surgical procedures for the cannulation of mesenteric lymph duct were adapted from those described by Raub et al. (1992) and Edwards et al. (2001). The stomach was cannulated by using a 3-cm piece of PE 50 tubing and was used for administration of the preparations as well as rehydration solution. Cyanoacrylate glue was used to secure the point of entry of the cannula.

The cefotaxime solution and liposomal formulation (each with a volume of 3.2 mL and containing 16 mg of cefotaxime) were administered intragastrically after successful cannulation of the mesenteric lymph duct was ascertained. Following administration, gentle massage of the stomach was performed to simulate the peristaltic movement and to ease the moving of the preparations to duodenum. The neck of the rat was tilted and supported by a 5-mL syringe to prevent the formulation from back flowing to the oesophagus. Blood samples were withdrawn at 0, 0.25, 0.75, 1.25, and 1.75 h postadministration. Approximately 0.5 mL of blood samples were collected from the tail vein into heparinized tubes. The blood samples were then centrifuged for 20 min at 12,800 rpm. About 0.2–0.3-mL aliquot of plasma obtained was transferred into a new microcentrifuge tube. Intestinal lymph samples were collected continuously for 0.5 h before and every half an hour for 2 h after dosing into heparinized tubes. The lymph flow was constantly monitored, and Ringer's solution was given to rehydrate the rat if necessary. All plasma and lymph samples were stored frozen at –20°C until analysis.

At the conclusion of the experiment, the animals were killed by using chloroform, and the integrity of the mesenteric lymph cannula was verified.

Data Analysis

Study I

The different preparations were compared by using the pharmacokinetic parameters, total area under the plasma concentration-time curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}), and time to reach peak plasma concentration (T_{max}). The C_{max} and T_{max} values were obtained directly from the plasma concentration data (Weiner, 1981), whereas $AUC_{0-\infty}$ was obtained by adding the area from time zero to the last sampling time (AUC_{0-t}) and the area from the last sampling time to infinity ($AUC_{t-\infty}$). The former was calculated by using the trapezoidal formula and the latter by extrapolating the last measurable plasma concentration until the time axis.

Study II

The ratios of cefotaxime concentrations in lymph over that of in plasma were compared for each of the time interval. Because cannulation of the mesenteric

lymph essentially collects all the lymph draining the small intestine, it was assumed that systemic plasma concentrations of cefotaxime reflected absorption of the drug via the portal blood. Therefore, the cefotaxime aqueous solution and liposomal formulation were compared by using the ratio of cefotaxime concentrations in lymph over plasma, which gives an indication of the contribution of lymphatic transport compared with portal route for the absorption of the drug.

Statistical Analysis

Study I

For the parameters $AUC_{0-\infty}$ and C_{max}, the values obtained were analyzed statistically by using an analysis of variance procedure (ANOVA) appropriate for a three-way crossover study that distinguishes effects due to group, subjects/group, period, and treatment (Wagner, 1975). The $AUC_{0-\infty}$ and C_{max} values were logarithmically transformed prior to the analysis. Should a statistically significant difference be obtained from the ANOVA procedure, the Tukey's test was used to compare between each pair of means of the three treatments. On the other hand, the T_{max} values were compared by using the Friedman test. A statistically significant difference was considered at $P < 0.05$.

Study II

The ratios obtained were analyzed statistically by using an analysis of variance (ANOVA) appropriate for a split-plot repeated measures design (Kirk, 1968). Should a statistically significant difference be obtained for main effects, tests for simple main effects were carried out to compare the effect of treatments at different time intervals as well as the effect of periods of time for a particular treatment. A statistically significant difference was considered at $P < 0.05$.

Assay Procedure for Cefotaxime Levels in Plasma and Lymph

Plasma and lymph cefotaxime levels were determined by using a HPLC method reported by Ling et al. (2003).

RESULTS

Particle Size of Liposomes

Table 2 shows the ZAve and polydispersity index of blank liposomes, cefotaxime-loaded liposomes, and the blank liposomes in the physical mixture. It can be inferred from the ZAve values that cefotaxime-loaded liposomes possessed bigger particle sizes than both blank liposomes and the liposomes in the physical mixture. When analyzed by using the ANOVA procedure followed by Tukey's test, a statistical significant difference ($P < 0.01$) was observed for ZAve between cefotaxime-loaded liposomes vs. blank liposomes as well as between cefotaxime-loaded liposomes vs. liposomes in the physical mixture. The ZAve value of the liposomes in the physical mixture was also slightly bigger ($P < 0.05$) than that of the blank liposomes.

TABLE 2 **ZAve and Polydispersity Index of the Preparations (Mean ± SEM, n = 18)**

Preparations	ZAve (nm)	Polydispersity Index
Blank liposomes	263.9 ± 2.1	0.28 ± 0.01
Physical mixture	275.6 ± 1.9	0.31 ± 0.01
Cefotaxime-loaded liposomes	326.4 ± 2.3	0.38 ± 0.01

The polydispersity index was found to increase in the following manner: blank liposomes < physical mixtures < cefotaxime-loaded liposomes. This indicates that the addition of the drug may affect the homogeneity of the size of liposomes produced. In all cases, the liposomes were in nanometer size range with narrow size distribution.

Entrapment Efficiency

There was negligible entrapment of cefotaxime by the liposomes in the physical mixture, with less than 2% of the drug being entrapped. In the drug-loaded liposomes, approximately 23.9 ± 1.0% of the cefotaxime was found to be located within the liposome vesicles, yielding an entrapment efficiency of 1.9 mg cefotaxime per 100 mg of phospholipid.

Animal Experiments

Study I

Mean plasma concentration vs. time profiles of cefotaxime aqueous solution, cefotaxime-loaded liposomes, and physical mixture of blank liposomes with cefotaxime solution are shown in Fig. 1. The cefotaxime-loaded liposomes showed a marked increase in oral bioavailability compared with that of cefotaxime aqueous solution or the physical mixture.

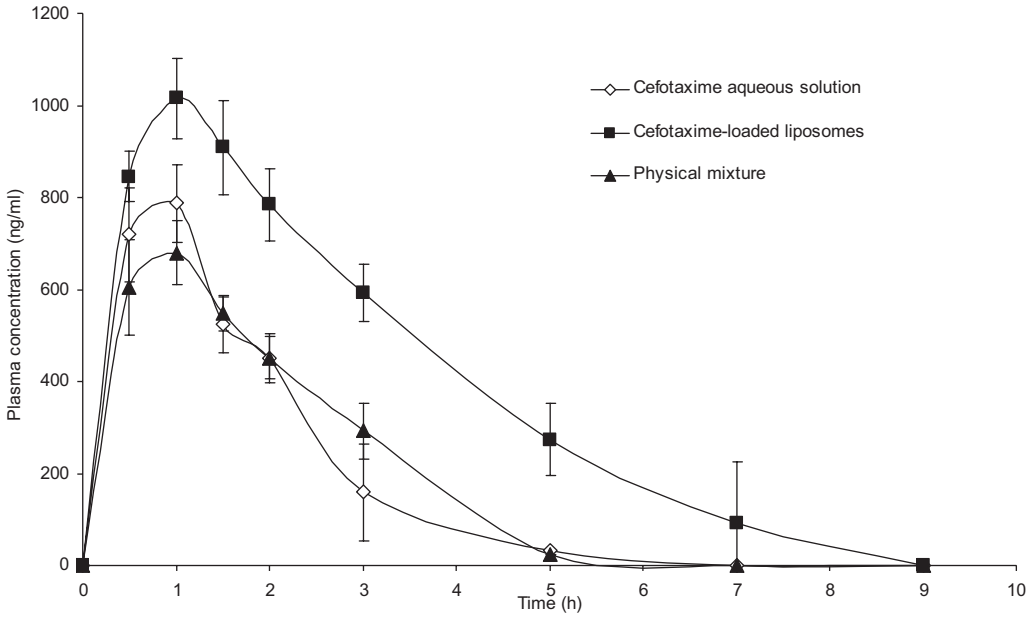

FIGURE 1 **Mean plasma concentration vs. time profiles of cefotaxime (mean ± SEM, n = 9) after oral administration of cefotaxime aqueous solution, cefotaxime-loaded liposomes, and physical mixture of blank liposomes with cefotaxime.**

Table 3 shows the individual numerical values of $AUC_{0-\infty}$ C_{max} and T_{max} obtained after oral administration of the three preparations. It can be seen from the table that the mean $AUC_{0-\infty}$ value of the liposomal formulation was almost twofold higher than that of the other two preparations, which have comparatively similar values. When the parameters obtained with the three preparations were analyzed by using the ANOVA procedure followed by Tukey's test, a statistical significant difference ($P < 0.01$) was observed for $AUC_{0-\infty}$ between cefotaxime-loaded liposomes vs. cefotaxime aqueous solution as well as between cefotaxime-loaded liposomes vs. the physical mixture. No statistically significant difference ($P > 0.05$) was observed for $AUC_{0-\infty}$ values between cefotaxime aqueous solution and the physical mixture. Thus, it is apparent that the bioavailability of cefotaxime was increased only when they were encapsulated in the liposomes, whereas mere mixing of the drug and the blank liposomes did not cause any significant increase in the bioavailability. The mean C_{max} value of the liposomal preparation was also markedly higher ($P < 0.05$) than that of the other two preparations. As for the cefotaxime aqueous solution and the physical mixture, the C_{max} values were quite similar and not significantly different statistically.

The 90% confidence interval of the $AUC_{0-\infty}$ and C_{max} values of the liposomal preparation over those of the other two preparations are shown in Table 4. It is apparent from the table that the extent of bioavailability of the liposomal formulation was about 1.7–3.0 times higher than that of cefotaxime aqueous solution and 1.6–2.8 times higher compared with the physical mixture. As for the C_{max} values, the 90% confidence interval showed that the peak concentration achieved with the liposomal formulation was about 1.3–1.8 times higher than that of the physical mixture and 1.1–1.5 times that of the aqueous solution.

In the parameter T_{max}, no statistical significant difference ($P > 0.05$) was found among the T_{max} of all three preparations. This suggests that the liposomal formulation only increased the extent but not the rate

TABLE 3 **Mean AUC0-∞, Cmax and Tmax for the Three Preparations (Mean ± SEM, n = 9)**

	$AUC_{0-\infty}$ (ng/mL × h)	C_{max} (ng/mL)	T_{max} (h)
Drug solution	1655.9 ± 229.4	858.7 ± 73.6	1.0 ± 0.2
Liposomal formulation	3589.8 ± 433.6	1067.0 ± 76.7	1.0 ± 0.1
Physical mixture	1739.7 ± 244.6	724.2 ± 73.6	0.9 ± 0.1

TABLE 4 **90% Confidence Interval Values**

	$AUC_{0-\infty}$	C_{max}
Liposomal formulation/drug solution	1.7–3.0	1.1–1.5
Liposomal formulation/physical mixture	1.6–2.8	1.3–1.8

of bioavailability of cefotaxime compared to the other two preparations.

Study II

Figure 2 shows the ratio of cefotaxime concentration in lymph over that of in plasma at various time intervals after administration of cefotaxime aqueous solution and cefotaxime-loaded liposomes. It is apparent from the plots that the concentration of cefotaxime in the lymph was consistently much lower than its concentration in the plasma when administered as an aqueous solution, with all the ratio values being less than unity. In comparison, the ratio values were consistently higher than unity when the drug was administered via the liposomal formulation. A statistically significant difference ($P < 0.05$) was found between the ratios obtained with the cefotaxime aqueous solution and those obtained with the liposomal preparation at all time points, providing evidence that the liposomal formulation promoted lymphatic transport of the drug compared with the aqueous solution.

DISCUSSION

The liposomes prepared with and without drug loading all possessed mean particle sizes in the nanometer range with narrow size distribution, as indicated by the values of ZAve and polydispersity index, respectively. The absorption efficiency in gastrointestinal tract has been shown to be affected by the size of the particles administered (Chen and Langer, 1998). Eldridge et al. (1990) reported that the extent of particle uptake in mice was size dependent, and particles greater than 10 μm were not taken up, whereas Desai et al. (1996) suggested that a size exclusion phenomenon exists in gastrointestinal absorption of particles, with smaller particles showing a significantly higher uptake than larger particles. Therefore, particle size is an important parameter to be monitored.

As much as 24% of the cefotaxime was entrapped in the liposomal formulation, whereas the physical mixture of cefotaxime with blank liposomes showed negligible entrapment. Negatively charged proliposome was chosen because it showed higher entrapment efficiency than neutral proliposome and also improved stability by preventing liposome aggregation (Betageri et al., 1993). The presence of electrostatically charged species increases the spacing between the phospholipid bilayers, causing an increase in the volume of the aqueous compartments and thus in the amount of hydrophilic drug entrapped (Alpar et al., 1981).

From Fig. 1, cefotaxime delivered as a liposomal formulation showed an increase in the extent of absorption as evidenced by the higher C_{max} and $AUC_{0-\infty}$ values compared with cefotaxime aqueous

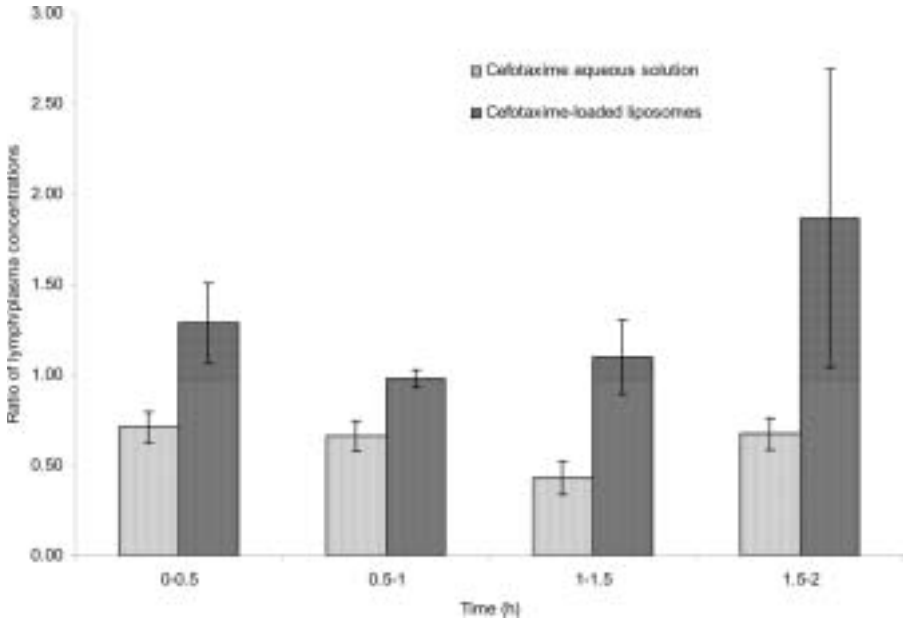

FIGURE 2 **Ratio of cefotaxime concentrations in lymph over that of in plasma vs. time profiles of rats (mean ± SEM, n = 10) after oral administration of cefotaxime aqueous solution and cefotaxime-loaded liposomes.**

solution or physical mixture. This suggests that cefotaxime entrapment in the liposomes is important for enhancing the oral bioavailability of the drug. The results obtained here are in accord with those reported by Ueno et al. (1982) and Kisel et al. (2001), in which the increase of plasma drug concentration or its biological activity was attributed to delivering the drug entrapped in liposomes and not by the mere addition of empty liposomes.

The low oral bioavailability of cefotaxime is mainly ascribed to its chemical instability in the stomach. Previous studies have shown that cefotaxime is hydrolysed in strong acidic pH (Berge et al., 1983; Gupta, 1984). Therefore, entrapment by liposomes might provide a temporary protection for the drug from the hostile acidic environment of the stomach. Besides, the dosing volume might infer buffering effect on gastric acidity, and hence reducing the instability of cefotaxime. In addition, lipid adjuvants such as phospholipids have been shown to exert a favorable effect on the absorption of drugs. This was demonstrated by Chandler et al. (1994) who reported that the increased nasal absorption of insulin coadministered with lysophosphatidylcholine was due to its surfactant property, which could cause moderate disruption of the nasal epithelium. However, in our study, an increase in the bioavailability of cefotaxime was not observed when it was given as a physical mixture with blank liposome compared with the cefotaxime aqueous solution. Thus, the enhanced oral bioavailability observed with cefotaxime-loaded liposomes was unlikely to be due to the increase in the intestinal permeability induced by the lipid components of liposomes.

Other mechanisms to explain the enhanced oral bioavailability of liposomally loaded drugs have also been postulated. Iwanaga et al. (1997) suggested that liposomes accumulated at brush-border membrane of enterocytes and increased the gradient of drug concentration across the intestinal epithelium, thus enabling the absorption of significant amount of insulin into the systemic circulation. A similar explanation was also given by Maincent et al. (1986) in the study of nanoparticles. Another potential mechanism is the uptake and transport of intact liposomes across the small intestine as evidenced by the study of Kimura (1988). The absorption of particulates in the intestine following oral administration is currently thought to occur via three possible mechanisms (Florence, 1997; Chen and Langer, 1998). Volkheimer (1977) suggested the intestinal uptake of particles in the micron size range occurred through the paracellular passage, and this phenomenon was termed "persorption." Other investigators suggested possible particle absorption by intestinal enterocytes through endocytosis (Sanders and Ashworth, 1961; Matsuno et al., 1983; Jani et al., 1992). Even though the uptake of particulates by enterocytes is not efficient, its contribution may be significant due to its large number present in the intestine. However, most of the studies suggested that the absorption of particulates predominantly takes place at the gut associated lymphoid tissue (GALT) involving isolated lymphoid follicles and Peyer's patches (LeFevre et al., 1978; LeFevre et al., 1985; LeFevre and Joel, 1986; Jani et al., 1989; Aramaki et al., 1993). The epithelial cell layer overlying the Peyer's patches contains specialized M cells. Although M cells occupy a small fraction of the intestine, it offers potential advantages over the enterocytes in the absorption of particulates. M cells have sparse, irregular microvilli on their apical surface in contrast to the rigid, closely packed microvilli typical of the enterocytes (Neutra, 1998; Kaiserlian and Etchart, 1999). Moreover, mucus is also present in reduced quantity, and the glycocalyx is relatively thin compared with the thick, continuous glycocalyx layer on enterocyte apical surfaces, which serves as a diffusion barrier that prevents direct contact of most particles with the microvillus membrane (Maury et al., 1995; Frey et al., 1996). These distinguish features render the M cell apical membrane more accessible to particulate absorption. While enterocyte brush borders have abundant hydrolytic enzymes, these enzymes are usually reduced or absent on M cells (Neutra, 1998). In addition, M cells contain few lysosomes and hence most particulate materials are trancytosed by M cells without degradation, unlike materials taken up by enterocytes which will be transferred to lysosomes where the contents will be subjected to enzymatic digestion (O'Hagan et al., 1992). Uptake of ferritin-containing polymerized liposomes by M cells through trancytosis has been observed with transmission electron microscopy (Chen and Langer, 1998).

Liposomes used in the present study possessed similar characteristics (negatively charged with mean diameter of 326.4 nm) with those studied by Tomizawa and coworkers (1993). In their in situ experiment, uptake of phosphatidylserine liposomes was greater in rat's Peyer's patch than in nonpatch tissue. In addition,

they also showed that negatively charged liposomes with a larger mean diameter (374 nm) yielded higher uptake. An earlier study by Patel et al. (1985) also reported a favorable transport of negatively charged liposomes across rabbit ileum compared with the neutral or positively charged liposomes. Thus, the enhanced bioavailability of liposomally loaded cefotaxime observed in the present study could in part be ascribed to the intestinal uptake of negatively charged liposomes by endocytosis via both Payer's patches and normal intestinal enterocytes.

It has been reported that particle uptake across the gastrointestinal tract occurs only to a very limited extent (LeFevre et al., 1985; LeFevre and Joel, 1986; Ebel, 1990; O'Hagan, 1990). Nevertheless, it could still contribute toward improvement in oral bioavailability of drugs such as cefotaxime that are negligibly absorbed if administered alone. Furthermore, entrapped drug could also be transported through the lymphatic system. As observed in the present study, the concentration of the drug in the lymph was consistently higher than that in plasma when it was given in the liposomal preparation. This provides evidence that liposomal entrapment could promote lymphatic transport of cefotaxime.

One of the factors favoring lymphatic transport is increasing molecular size. Lymphatic capillaries are different from blood capillaries in which the former consists of a single layer of nonfenestrated endothelial cells that lack a continuous basal lamina. Besides, there are gaps between intercellular junctions ranging between 15 nm to several microns (O'Driscoll, 1992). In contrast, blood capillaries in small intestine consist of fenestrated endothelial cells with continuous basement membrane. Studies on the permeability of intestinal capillaries to endogenous macromolecules have shown that these capillaries selectively restrict macromolecules to a greater degree than the continuous type found in other organs (Granger and Taylor, 1980; O'Driscoll, 1992). For example, chylomicron, the largest (75–600 nm) lipid-carrying particles in the lymph, is too large to enter directly the fenestrated blood capillaries of the mucosa and hence are transported via the lymph (O'Driscoll, 1992). Thus, entrapping hydrophilic drugs in particulates such as liposomes might promote their transport via the lymphatic system on absorption due to the size of the particulate carrier. In the present study, lymphatic transport of cefotaxime was observed when administered via the liposomes. However, it might not have major contribution to the increased bioavailability of the drug because its concentration in the lymph was only about two fold higher than that in the plasma. Moreover, the lymphatic flow rate is markedly lower than that of the hepatic vein flow rate. Nevertheless, the results demonstrated that the liposome system could promote the lymphatic transport of a hydrophilic drug.

CONCLUSION

In summary, the liposomal formulation was able to enhance the oral bioavailability of cefotaxime, and liposome entrapment appeared to play a significant role in enhancing the oral bioavailability of poorly absorbed hydrophilic drugs. Moreover, the liposome system could promote the lymphatic transport of the hydrophilic drug although this might not have major contribution to the observed increase in bioavailability of the drug.

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