Formulation and Evaluation of a Folic Acid Receptor-Targeted Oral Vancomycin Liposomal Dosage Form

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Purpose. To demonstrate utility of folic acid-coated liposomes for enhancing the delivery of a poorly absorbed glycopeptide, vancomycin, via the oral route.

Methods. Liposomes prepared as dehydration–rehydration vesicles (DRVs) containing vancomycin were optimized for encapsulation efficiency and stability. A folic acid-poly(ethylene oxide)–cholesterol construct was synthesized for adsorption at DRV surfaces. Liposomes were characterized by differential scanning calorimetry (DSC) and assessed *in vitro* in the Caco-2 cell model and *in vivo* in male Sprague–Dawley rats. Non-compartmental pharmacokinetic analysis of vancomycin was conducted after intravenous and oral administration of solution or liposome-encapsulated vancomycin with or without 0.05 mole ratio FA-PEO-Chol adsorbed at liposome surfaces.

Results. Optimal loading of vancomycin (32%) was achieved in DRVs of DSPC:Chol:DCP, 3:1:0.25 mole ratio (m.r.) after liposome extrusion. Liposomes released less than 40% of the entrapped drug after 2 hours incubation in simulated gastrointestinal (GI) fluid and simulated intestinal fluid containing a 10 mM bile salt cocktail. Incorporation of FA-PEO-Chol in liposomes increased drug leakage by 20% but resulted in a 5.7-fold increase in Caco-2 cell uptake of vancomycin. Liposomal delivery significantly increased the area under the curve of oral vancomycin resulting in a mean 3.9-fold and 12.5-fold increase in relative bioavailability for uncoated and FA-PEO-Chol-coated liposomes, respectively, compared with an oral solution.

Conclusions. The design of FA-PEO-Chol-coated liposomes resulted in a dramatic increase in the oral delivery of a moderate-size glycopeptide in the rat compared with uncoated liposomes or oral solution. It is speculated that the cause of the observed effect was due to binding of liposome-surface folic acid to receptors in the GI tract with subsequent receptor-mediated endocytosis of entrapped vancomycin by enterocytes.

KEY WORDS: folic acid; liposomes; vancomycin; oral targeted delivery; Caco-2.

INTRODUCTION

The extent of systemic availability of proteins/peptides achieved thus far has been the direct result of multidisciplinary efforts to commercialize this potent class of pharmaceutical compounds into readily usable dosage forms. Only limited success has been obtained using orally delivered proteins/peptides (e.g., desmopressin, cyclosporin), whereas important advances in oral protein/peptide vaccines have been possible (1). In recent years, a greater understanding of gastrointestinal (GI) physiology and uptake mechanisms and promotion of the oral delivery of colloidal dosage forms have led to a number of novel concepts for oral protein/peptide delivery (2–8).

Oral liposome drug delivery has not been accepted widely because results have been quite variable and, for the most part, not predicated on specific objectives that would lead to success (8). Successful delivery of oral protein/peptide liposome systems depends on formulation strategies designed to achieve good intestinal stability (of liposome and drug), greater GI wall affinity, and ample protein/peptide loading at the site of delivery.

The potential use of cellular nutrients as mediators of liposomal uptake is enticing because receptor-mediated endocytosis is a cellular process designed for transporting critical molecules across the plasma membrane into the cytoplasm (4,9). Folic acid is an essential nutrient required for nucleotide synthesis and is absorbed by way of a saturable, pH-dependent, sodium ion-dependent, and metabolic inhibitor-sensitive pathway (9) in addition to being predisposed to potosomal cytoplasmic routing (10). The specificity and pH dependence of folate absorption from the GI tract have been described (11), leading to the possibility of using this pathway for promoting folate-linked liposomes to enter GI epithelia. Evidence of folate-mediated uptake of macromolecular conjugates, microparticulates, and liposomes support the proposed utility and functionality of this cellular routing (12–14). The Caco-2 cell model is regarded as a useful tool for assessing potential candidates for GI absorption, including colloids, and expresses folic acid-binding proteins (15,16).

Vancomycin (VCM), a glycopeptide antibiotic, was chosen as a model for peptide absorption because of its physicochemical properties, including a molecular weight greater than the cut-off for paracellular transport (1449.2), multiple anionic and cationic charged groups (pI 7.0), and high hydrophilicity ($S_o \approx 15$ mg mL^{-1} at pI, RT). VCM solubility ranges from 15–100 mg mL^{-1} depending on the pH of the environment. VCM is poorly absorbed from the GI tract (bioavailability <2%) and undergoes clearance predominately by the kidneys (>95%), thus providing an excellent model for assessing the effect of liposomes on the GI transport of proteins/peptides without the confounding variable of first-pass metabolism as occurs with many other bioactive agents (e.g., insulin) (17). This study was intended to demonstrate feasibility of oral folic acid-mediated liposomal delivery of a model peptide.

MATERIALS AND METHODS

Materials

Distearoylphosphatidylcholine (DSPC) and dimyristoylphosphatidylglycerol (DMPG, Na salt) were obtained from Princeton Lipids (Princeton, NJ). Cholesterol (Chol), dicetylphosphate (DCP), sodium cholate (NaC), sodium chenodeoxycholate (NaCDC), sodium deoxycholate (NaDC), VCM, poly(ethylene(bis-amine)oxide) (PEO, nominal MW

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3350), pteroylglutamic (folic) acid (FA), dicyclohexylcarbodiimide, and cholesterol chloroformate were obtained from Sigma Chemical Co. (St. Louis, MO). Cetyltrimethylammonium bromide (CTAB) was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Reagent-grade DMSO, DMF and pyridine were distilled before use.

Folic Acid-poly(ethylene oxide) (FA-PEO) Conjugates

The synthesis of FA-PEO conjugates was performed at a 1:1 mole ratio (m.r.) of FA and PEO under previously described conditions (18), dialyzed against 5 mM borate buffer (pH 8) for 3 days (Spectra/Por® 3000 MW dialysis membrane, Spectrum, Houston, TX) and further purified by batch adsorption with cellulose–phosphate resin in 5 mM phosphate buffer (pH 7.0) to remove unreacted PEO. The suspension was filtered to remove the resin and the clear filtrate was lyophilized for 12 hours. Chol was conjugated with FA-PEO (FA-PEO-Chol, FPC) by reacting a 10-fold molar excess of cholesterol chloroformate with FA-PEO solubilized in dry DMF and dry DMSO, respectively, in the presence of dry pyridine for 24 hours at room temperature in the dark. The solution was dialyzed against pure DMSO (Spectra/Por® 3000 MW dialysis membrane) for 24 hours. The dialyzed solution was filtered (0.22 μm) to remove unreacted Chol and the filtrate was lyophilized. The identity and *in vitro* performance of this construct has been confirmed elsewhere (18).

Liposome Preparation

DSPC, Chol, DMPG, and DCP were dissolved in 1:9 (v/v) methanol:chloroform and rotary evaporated to form a thin lipid film on the walls of a round-bottom flask. Mole ratios of the negatively charged lipids (DMPG, DCP) were varied, while the DSPC:Chol m.r. was held constant at 3:1. Multi-lamellar vesicles (MLVs) were prepared by film hydration with an aqueous solution of VCM at either pH 4 (Walpole acetate buffer, 16.6 mM sodium acetate, 83.4 mM acetic acid) or pH 7 (Sørenson's phosphate buffer, SPB, 27.5 mM monopotassium phosphate, 39.2 mM dibasic potassium phosphate) to a final lipid concentration of 35 mM. Liposomes were prepared by hydrating the lipid film with double-distilled water and probe sonicating the 35 mM lipid suspension (W-375 ultrasonicator, Heatsystems-Ultrasonics, Plainview, NJ) at 55 °C for 5 minutes, resulting in a translucent aqueous dispersion of small vesicles (SUVs). The lipid dispersion was flash frozen and lyophilized overnight then dehydration–rehydration vesicles (DRVs) were prepared by adding 500 μL of a concentrated solution of VCM in SPB (pH 7.0) to the lyophilized lipids and vortexing. The gel produced was then diluted to 35 mM with 4.5 mL SPB. The DRVs were serially sized by individual passes through 1.0 μm, 0.8 μm, 0.4 μm, and 0.2 μm polycarbonate membranes (Lipex® Extruder, Lipex Biomembranes Inc., Vancouver, Canada) at 65 °C. Size analysis of the resulting liposomes was performed using a BI-90 laser light scattering particle sizer (Brookhaven Instruments Corp., Holtsville, NY). Briefly, samples of liposomes (50 μL) were diluted to 3 ml with SPB before analysis at 5 mW using a HeNe laser vertically polarized. Finally, gel permeation chromatography (GPC, Sephadex G-50, 1.5 × 15 cm) was used to separate free and liposome-encapsulated VCM.

DRVs of DSPC:Chol:DCP prepared as described were vortex mixed with FA-PEO-Chol, yielding FA-coated liposomes of DSPC:Chol:DCP:FA-PEO-Chol (3:1:0.25:0.05, m.r.).

DRV formulations for the *in vivo* studies were prepared using the same drug loading but at 3-fold higher lipid concentrations.

Encapsulation Efficiency

Liposomes were solubilized in 20 mM CTAB solution for 5 minutes at 60 °C prior to analyzing for VCM content. Encapsulation efficiency (EE) of VCM in liposomes was determined as follows:

$$\%\,\mathrm{EE} = \frac{C_{GPC}}{C_{orig}} \times 100, \quad (1)$$

where C_{GPC} is the concentration of VCM in the liposome dispersion (accounting for GPC dilution if necessary) and C_{orig} is the original concentration of VCM added.

Drug Release

Release of VCM from liposomes was assessed by dialyzing a 0.5–1.0 mL aliquot of VCM liposomes (Spectra/Por®, 10,000 MWCO. dialysis membrane) against 50 ml of freshly prepared USP-simulated gastric fluid (pH 1.2 without pepsin, SGF), USP-simulated intestinal fluid (pH 7.5 without pancreatin, SIF), SIF with 10 mM NaC (SIF+), or SIF with a 10 mM bile salt cocktail of NaC, NaCDC, and NaDC (2:2:1 m.r.) (SIF + BSC) at 37 °C under moderate magnetic stirring conditions then measuring the dialysate for the presence of VCM over a period of 2 hours. Average bile acid concentrations of 10 mM are considered to be similar to human intestinal bile concentrations, although bile acids are generally conjugated to glycine or taurine *in vivo* (19).

Differential Scanning Calorimetry (DSC)

Liposomes were analyzed by DSC (SSC/5200 SII DSC 120, Seiko Instruments Corp.) to assess the effect of lipid composition, and VCM or FA-PEO-Chol inclusion on the phase transition temperature (T_m) of native DSPC liposomes. Thermograms were obtained by heating samples at 2 °C/minutes from 25–100 °C. Thermal data analysis was carried out with a HP 9000 series 700 workstation (Hewlett Packard, Palo Alto, CA) equipped with HP VUE® and a DSC module.

Caco-2 Cell Culture

Caco-2 type BBE_1 cells (a gift from Dr. M. Mooseker, Yale University) were grown in folate-free Dulbecco's Modified Eagles Medium (FFDMEM), supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, and 0.002% insulin. Cells were seeded at 2×10^6 cells between passages 79–81 on high-density, collagen-coated, 1.0-μm pore-size Falcon PET inserts (24 mm, Becton Dickinson, Oxford, UK) and used between days 7–10 for transport studies. Cells attached to filters were fed daily with 10% FBS-supplemented FFDMEM for days 6–9 and fed FA-depleted media for 1 day before transport studies. FA-depleted media consisted of FFDMEM supplemented with 10% FBS that was pretreated

with activated charcoal to remove endogenous folates (20). Tight junction formation was monitored by immunofluorescent labeling of the tight junction protein ZO-1. Integrity of cell monolayers was confirmed by transepithelial electrical resistance (TER). Transport studies were conducted in Hepes-buffered saline with 1 g/L glucose, 1.3 mM $CaCl_2$, and 0.5 mM $MgCl_2$ (HBSG+) at pH 5.8 (optimal pH for folate uptake) for a 2-h period. Apparent permeabilities (P_{app} = transepithelial flux) of the cells to VCM, liposomal VCM, and FA-coated liposomal VCM were calculated according to:

$$P_{app} = \left(\frac{V}{A \cdot C_o}\right) \times \frac{dC}{dt} \quad \text{(cm/s)}, \tag{2}$$

where $V \cdot (dC/dt)$ is the steady-state rate of appearance of apically-applied conjugates in the basal medium, C_o is the initial conjugate concentration in the apical medium, and A is the monolayer area.

In Vivo Protocol

Male Sprague–Dawley rats (295.4 ± 13.2 g) were obtained from the Biosciences Animal Service (University of Alberta, Edmonton, AB, Canada) and housed for at least 2 days in a clean room with access to food and water ad libitum. One day before the experiment, anesthesia of the rat was induced by pentobarbital (65 mg kg^{-1}) and a small incision was made over the right jugular vein. The vein was catheterized with Silastic® laboratory tubing (0.635 mm i.d., 1.194 mm o.d., Dow Corning Corp., Midland, MI) containing heparinized (100 IU mL^{-1}) normal saline and fixed in place with two nonabsorbable surgical sutures (Surgical Suture USP, Cyanamid, Montreal, QC, Canada). Each cannula was terminated with a long piece of polyethylene tubing (PE-50, i.d. 0.58 mm, o.d. 0.965 mm, Clay Adams, Parsippany, NJ) and the free end exteriorized to the dorsal side of the neck. The rats were fasted while recovering from surgery for at least 16 hours and allowed access to water ad libitum. Group I was administered an i.v. solution of VCM in Hepes-buffered saline via the jugular vein cannula (6.85 mg kg^{-1}) and 0.25-ml blood samples were drawn at times –0.5, 2, 5, 15, 30, 45, 60, 90, 120, and 180 minutes post-administration. Groups II, III, and IV were administered orally a solution of VCM in Hepes-buffered saline (61.75 mg kg^{-1}), a suspension of uncoated liposome-entrapped VCM (62.4 mg kg^{-1}), or a suspension of FPC-coated liposome-entrapped VCM (47.69 mg kg^{-1}) via an oral gavage tube. Subsequently, 0.25-ml blood samples were drawn at –0.5, 15, 30, 60, 90, 120, 180, 240, 300, and 360 minutes post-administration. Blood samples were immediately centrifuged at 3400 g for 5 minutes and the plasma was collected and stored at –20 °C. Long-term VCM stability (>1 year) in serum at –20 °C has been demonstrated previously (21). The total blood volume drawn from each rat represented approximately 12.5% of the blood volume available. The total fluid volume administered was 0.2 ml for i.v. dosing and 2.0 ml for oral dosing. The total lipid load in oral dosing of liposomes was approximately 0.30 mmol. The Guide to the Care and Use of Experimental Animals of the Canadian Counsel on Animal Care was followed throughout.

Chromatographic Analysis

In Vitro

High-performance liquid chromatography (HPLC) coupled with UV detection (Lambda-max model 481, Waters, Milford, MA) at 229 nm was used to quantitate VCM in samples. The mobile phase was prepared by premixing SPB (pH 7):acetonitrile (95:5 v/v), degassed, and pumped (Model 501 HPLC pump, Waters) through a cyano (-CN) column (Radial Pak cartridge, 8 × 10 RCM, Waters) at a flow-rate of 1 ml/min. All reagents were HPLC grade. VCM samples were diluted as necessary and 100-μl samples were injected on to the column directly via an auto sampler (712 Wisp, Waters). Samples containing liposome dispersions were pretreated in 20 mM CTAB at 60 °C to solubilize liposomes before VCM analysis. The concentration of VCM was calculated from a calibration graph (peak area vs. concentration).

In Vivo

HPLC coupled with UV detection (Gilson 117, Middleton, WI) at 250 nm was used to quantitate VCM. Mobile phase of SPB (pH 7.0):acetonitrile (9:1, v/v) was degassed and pumped through a 5-μm reverse-phase C-8 column (124 × 4 mm LiChrospher 60 RP-Select B, Merck, Germany) at a flow-rate of 1 mL/min. All reagents were HPLC grade.

Before analysis, plasma samples (100 μl) were treated with 300 μl 10% TFA:methanol (2:1, v/v), vortexed, and centrifuged at 14000 rpm for 10 min (Eppendorf 5415 microcentrifuge, Germany) to remove plasma proteins. The supernatant was collected and fresh buffer solution was added to a total volume of 500 μl (4-fold sample dilution). Samples (100 μl) were injected by an auto sampler (Basic Marathon, Spark Holland, Netherlands). Peak areas of VCM were quantitated using the Data Ally™ (Lab Alliance, PA) data acquisition program. VCM concentrations were calculated from a standard calibration curve (2–50 μg ml^{-1}) prepared daily from VCM-spiked rat plasma. No interference by plasma components was detected. The limit of VCM detection in plasma was 2 μg ml^{-1}, in agreement with other reports (17,21,22).

Pharmacokinetic Analysis

Standard pharmacokinetic parameters were obtained from individual rat plasma concentration–time profiles of VCM calculated according to the standard non-compartmental analysis in WinNonlin Standard Edition version 1.0 (SCI, Apex, NC). Area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule. Absolute bioavailability (F) was calculated according to:

$$F = \frac{AUC_{test}^{0\text{–last}}}{AUC_{iv}^{0\text{–last}}} \times \frac{Dose_{iv}}{Dose_{test}} \tag{3}$$

where AUC_{test} and $Dose_{test}$ represent the respective mean oral dosing parameters of individual groups.

Statistical Analysis

Pharmacokinetic parameters were assumed to follow a log-normal distribution (except for t_{max}), and were log-transformed before statistical analysis. All parameters were recorded as arithmetic means (±SD). The area under the

curve (AUC) and C_{max} were dose-normalized before statistical analysis. The pharmacokinetic parameters obtained were analyzed by independent measures one-way analysis of variance, and a *post-hoc* Student–Newman–Keuls (SNK) test was used to determine where, if any, differences occurred. A value of $P \leq 0.05$ was considered statistically significant. The computer program SigmaStat 2.0 (SPSS, Chicago, IL) was used for all statistical procedures.

RESULTS AND DISCUSSION

The encapsulation of a water-soluble agent in liposomes is commonly correlated with the concentration of encapsulated aqueous volume and any electrostatic effects prevailing. Thus, in the case of VCM, the degree of encapsulation can be controlled by varying the liposome composition and the method of preparation. Table I summarizes the results obtained showing a range of %EE from 4–97%. It is apparent that the encapsulation was maximized using the DRV method of preparation compared with the classical MLV method, overshadowing any influence of electrostatic effects resulting from addition of a negatively charged component to the bilayers or a change in the pH. At pH 4, the %EE was drastically reduced in DRVs when DMPG, but not DCP, was incorporated. Thus, only by using a method of rapid hydration of the lipids using a concentrated solution of drug during liposome formation, such as accomplished by the DRV method, can any appreciable drug loading of a water-soluble glycopeptide be obtained. Extruding the DRVs (0.22 μm) substantially reduced the %EE. The average sizes of uncoated and FPC-coated liposomes after extrusion were 208.8 ± 8.9 and 291.7 ± 20.0 nm, respectively.

Evidence of low interaction of VCM with the liposomal bilayers was obtained from DSC measurements. Figure 1 illustrates only a slight reduction of the T_m of the DSPC bilayer due to VCM with an unchanged endothermic peak area. Typically, the inclusion of 25% Chol eliminated the melting isotherm of DSPC such that after the inclusion of Chol, DMPG, DCP, VCM, or FA-PEO-Chol the thermogram essentially was independent of temperature. Varying the initial VCM concentration in preparing DSPC:Chol:DMPG or DSPC:Chol:DCP (3:1:0.25 m.r.) liposomes had an insignificant effect on the %EE (results not shown), suggesting that electrostatic interaction of VCM with the bilayers accounted for a negligible amount of the total uptake. The concentration of VCM did not significantly alter the osmolarity of the loading solution.

Table I. Encapsulation Efficiency (%EE) of VCM in Liposomes as a Function of Composition, pH, and Method of Preparation[a]

Liposome composition[b]	%EE		
	MLV	DRV	Extruded DRV[c]
DSPC	—	58	—
DSPC:Chol	10	15	—
DSPC:Chol:DMPG	8	85	6
DSPC:Chol:DMPG (pH 4)	7	15	—
DSPC:Chol:DCP	4	97	33
DSPC:Chol:DCP (pH 4)	12	87	—

[a] Means ± SD, $n \geq 3$.
[b] VCM was loaded at pH 7 unless otherwise specified. DSPC:Chol mole ratio was fixed at 3:1 and DMPG or DCP was 0.25.
[c] 0.2 μm filters.

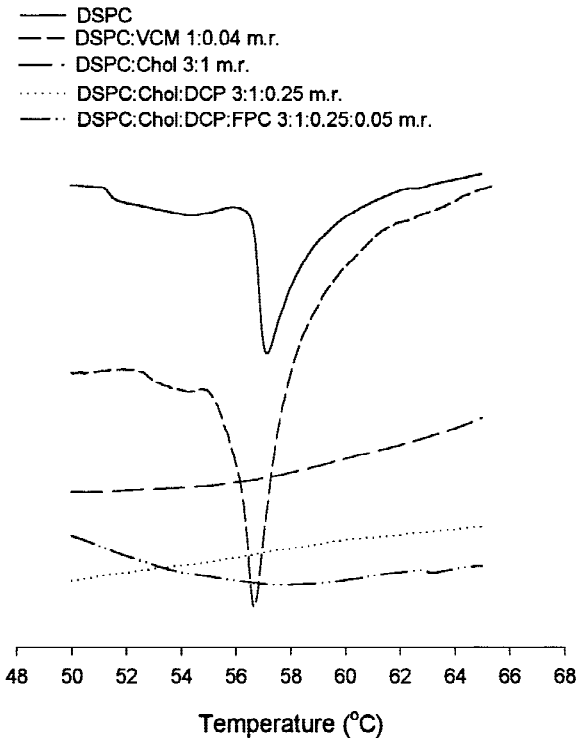

Fig. 1. DSC thermograms depicting different lipid compositions showing the effect of VCM and Chol on the gel–lipid crystalline phase transition (T_m) of DSPC liposomes.

The results of *in vitro* release of VCM from liposomes after a 2-hour incubation period in simulated GI fluids at 37 °C are presented in Figure 2. The liposome formulations were relatively stable to pH change and to the addition of bile salts, including the 10 mM bile salt cocktail. Under these relatively harsh conditions only up to 40% of the drug was released. Liposomes that had not been extruded appeared to be somewhat leakier, possibly because of weakly associated surface-adsorbed VCM. Although the addition of FA-PEO-Chol resulted in some drug release, the total amount of drug retained was >60% after a 2-hour incubation period in SIF + BSC. Liposomes containing either DMPG or DCP behaved similarly after the addition of FA-PEO-Chol.

The principal destabilizing influences of oral liposomes are pH and bile salts, pancreatic enzymes (phospholipases) being only a moderate influence. Liposomes administered orally should be able to resist solubilization by approximately 10 mM bile salts, which is more likely when they are multilamellar (MLVs or DRVs) rather than unilamellar (SUVs or LUVs) because a critical solubilizing ratio, R_e^c, must be obtained (23). There are several reports that demonstrate the instability of liposomes in bile salt solutions (9), particularly fluid-state liposomes (i.e., at temperatures $>T_m$ of the PC). In contrast, the results presented above and earlier reports (24) with DSPC/cholesterol liposomes provide evidence of the feasibility of formulating liposomes having adequate stabilities against bile-salt or phospholipase-induced leakage.

Extruded surface-adsorbed FA-PEO-Chol liposomes (~200 nm) retained at least 65% VCM after a 2-hour incubation in SIF + BSC. This same liposome composition had been

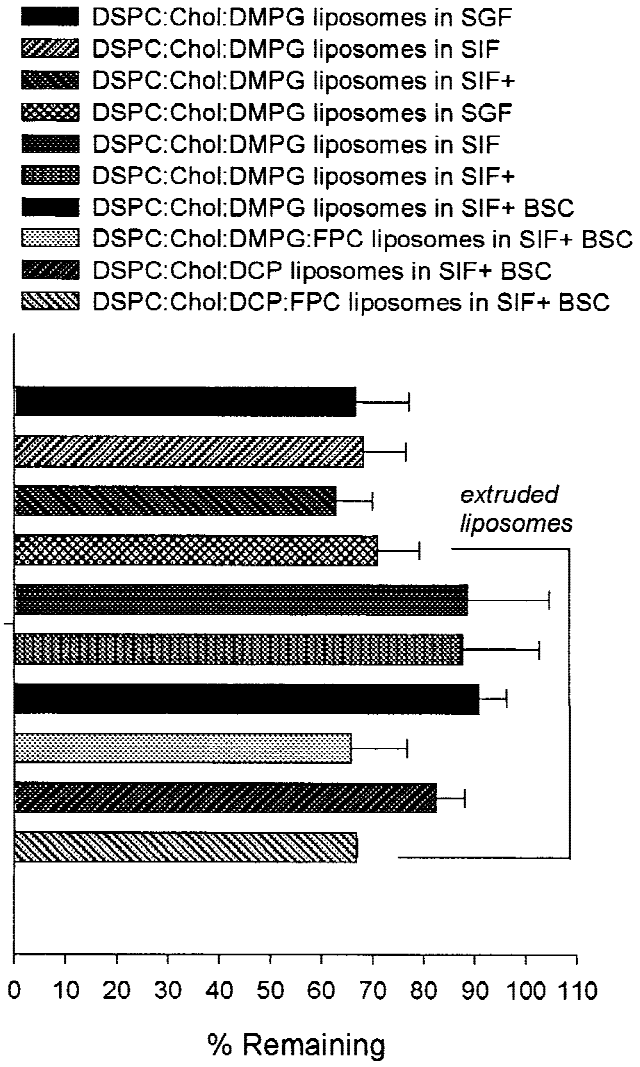

Fig. 2. *In vitro* release studies of VCM liposome formulations in simulated GI fluids. Data represents the mean % liposome-associated VCM retained after 2-hour dialysis at 37 °C (±SD, $n \geq 3$). Lipid compositions were DSPC:Chol:X 3:1:0.25 m.r., where X represents the negatively charged lipid component (DMPG or DCP). FA-PEO-Chol (FPC) was loaded at 0.05 m.r. where indicated. Liposomes were terminally extruded through 0.2-μm filters where indicated.

identified previously as having potential for improving the transepithelial flux of liposomes across GI epithelia (18). Table II provides evidence of VCM and liposomal VCM interaction with the Caco-2 cell monolayer. Integrity of the monolayer was established by immunofluorescent labeling of the ZO-1 tight junction protein and by TER values of 562 ± 93 $\Omega \cdot cm^2$. The rate of transport of VCM across the monolayers was not quantifiable over the 2-hour study period for all formulations tested. However, total recovery of VCM from basal and apical chambers indicated a substantial loss to the cells of VCM (~14%) after administration of FPC-coated liposomes, suggesting that cellular uptake and intracellular trapping or metabolism of VCM had occurred. Previous work employing the large MW, hydrophilic, fluorescent Texas Red Dextran (3000 MW) as a marker in FPC-coated liposomes yielded similar results (18), although Texas Red Dextran is a neutral molecule. Evidence of the implication of the FA receptor is the reversal of binding and uptake of the marker when free FA was included (see Table II, ref. 18). Experiments using Caco-2 monolayers characteristically are limited to low absorption surface areas and large TERs compared with normal intestinal epithelia. The poly(ethylene terphalate) membrane filters of the monolayer platform also presented a barrier to liposome transport thereby preventing critical assessment of the effects of the target liposomes on VCM transport. Nevertheless, noticeable cellular uptake of VCM associated with FPC-coated liposomes was a positive indicator of its potential as a delivery system. Whether VCM was metabolized or simply trapped intracellularly resulting in reduced transepithelial flux of VCM is unclear. A better understanding of enzymatic barriers to liposomes, liposome-associated molecules and cellular processing should help to expedite the design of oral liposomes for therapeutic use. Until better *in vitro* models are designed for assessment of transepithelial colloid transport, pre-clinical animal investigations are still necessary.

Table II. Transport of Liposomal VCM Across Caco-2 Monolayers

VCM dosage form	P_{app} (cm/s)	Percent loss across monolayer[a]
Solution, HBS pH 5.8	n.q.[b]	2.41 (1.07)
Uncoated liposomes	n.q.	–4.60 (3.62)[c]
FPC-coated liposomes	n.q.	13.83 (0.94)

[a] Mean (±SD, $n = 4$).
[b] Non-quantifiable.
[c] Negative numbers suggest concentration of VCM in apical chamber.

The pharmacokinetic parameters generated from non-compartmental analysis of *in vivo* data are given in Table III. Except for T_{max} statistical significance was found in all cases (log transformed data). After i.v. administration, VCM exhibited two-compartmental kinetics with a rapid distribution phase, a terminal half-life of approximately 50 min, and a total body clearance of 1.13 mL min^{-1} kg^{-1} in agreement with previous reports (17,25). The mean plasma concentration-time profiles obtained from all formulations are illustrated in Figure 3. Dose-normalized AUCs from time 0 to the last time point were utilized for bioavailability determinations. The *in vivo* profiles were characterized by multiple peaks which made it difficult to calculate AUCs from time 0 to infinity. The ratio, AUC/D, was significantly different for all formulations tested ($P < 0.05$). The absolute bioavailability calculated from the mean was 1.74 percent, in agreement with previous reports (17). The bioavailability of VCM from uncoated liposomes was 6.7%, a 3.9-fold increase, and 21.8% from FPC-coated liposomes, a 12.5-fold increase, each compared to an oral VCM solution. Thus, the folic acid marker was 3.2 times as effective as the uncoated liposome system.

An explanation of the multiple peaking profiles exhibited by the liposome systems is not immediately forthcoming. The larger magnitude of ensuing peaks is suggestive of a pre-absorptive phenomenon possibly related to delayed gastric emptying as a result of lipid dosing or complexation of VCM with DCP and partial precipitation at low pH. Regardless, FPC-coated liposomes significantly increased (3.2-fold) the delivery of VCM compared to uncoated liposomes even though the loading dose was 24% lower. Enterohepatic recir-

Table III. Pharmacokinetic Parameters of VCM After Intravenous (i.v.) and Oral Dosing

PK parameters	i.v.[a] (I)	Soln[a] (II)	Uncoated liposomes[a] (III)	Coated liposomes[a] (IV)	Statistical comparisons[b]
$AUC_{0\text{-last}}/D$ (mL^{-1} min kg^{-1}) ($\times 10^1$)	6.42 (1.05)	0.11 (0.04)	0.43 (0.27)	1.40 (0.86)	I II III IV
C_{Max} ($\mu g\ mL^{-1}$)	325.11 (38.00)	11.35 (2.61)	25.14 (13.06)	42.21 (21.96)	I II III IV
t_{max} (min)	0	80 (17.32)	210 (114.89)	112.5 (61.85)	II III IV

[a] Mean ± (SD, $n = 4$).
[b] All numbers were log-transformed before statistical analysis (with the exception of t_{max}). Formulation numbers underlined are not significantly different (one-way ANOVA, SNK, $P < 0.05$).

culation of liposomes or lymphatic absorption via Peyers patches are less likely causes of the observed multiple peaking. No physicochemical or physiological property of VCM per se would appear to be responsible for this behavior based on established testing (26). Maitani *et al.* (27) have suggested that liposomes may become trapped at pre-systemic sites after oral administration providing sustained release of entrapped protein.

Transport of liposomes into enterocytes via foliate receptor-mediated endocytosis could have functional therapeutic possibilities for protein/peptide delivery analogous to cyanocobalamin-mediated uptake of microparticulates (28). Targeted delivery of liposomes to receptor sites in the gastrointestinal tract may, indeed, be more beneficial than nonselective stable liposomes, such as polymerized liposomes (29), or relying on Peyer's patch uptake and delivery via the lymphatic system (8,30).

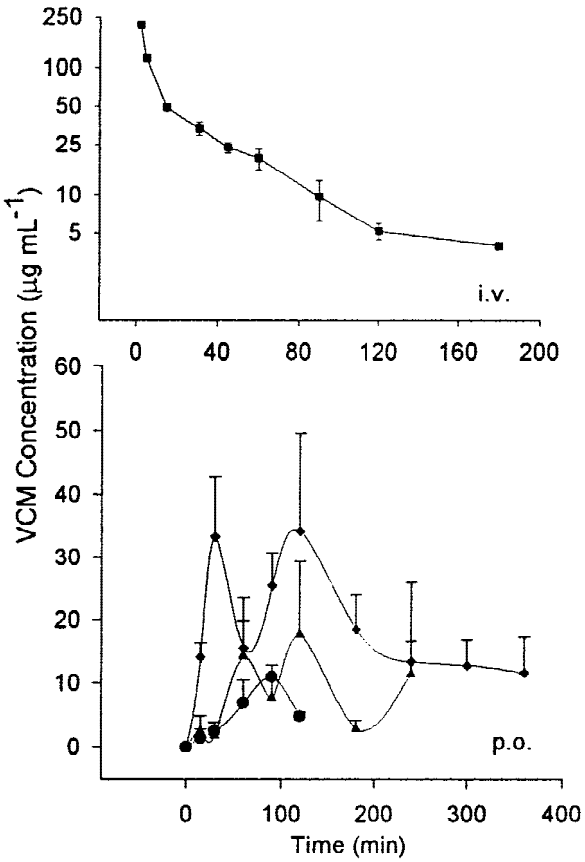

Fig. 3. *In vivo* disposition of VCM after i.v. administration (■) and oral administration of FA-PEO-Chol liposome-entrapped VCM (♦), uncoated liposome-entrapped VCM (▲), and VCM solution (●). Error bars represent standard deviations ($n = 4$).

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