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Liposomal formulations of ABT-077: In vitro characterization studies

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Abstract

ABT-077 (zileuton) is an active 5-lipoxygenase inhibitor with potential for the treatment of asthma and rheumatoid arthritis. It is rapidly eliminated after local administration to the lungs. Hence, various liposomal formulations of this compound were tested in vitro for potential modulated release in vivo. Fourteen ABT-077 compositions were screened for distribution coefficients (K) between lipid and aqueous buffer (0.05 M phosphate, pH 6.5). With lipids consisting of 20–50% w/w cholesterol (CHL) in egg phosphatidylcholine (PC), log K of approximately 2.15 and drug loadings of approximately 6 μg/mg were obtained. When the lipid film was washed with aqueous buffer, 60–80% of the originally loaded drug was retained. Thereafter, a 3^3 factorial study was conducted to investigate the effect of lipid composition (9, 35 or 50% w/w CHL in PC), drug level (1, 13 or 25% w/w of total lipid) and extent of dilution (0, 1:25 and 1:100 fold) on drug encapsulation in liposomes. The log K values ranged between 2.2 and 3.2, and drug loadings ranged between 6 and 230 μg/mg lipid. With diluted samples, estimated drug loadings ranged between 1 and 45 μg/mg lipid. Overall, the data suggests that encapsulation of ABT-077 may enable modulated drug release kinetics in vivo.

Keywords: Cholesterol; Phosphatidylcholine; Formulation optimization

1. Introduction

Leukotrienes, 5-lipoxygenase products of arachidonic acid metabolism, are generated from a wide variety of cells in the airways such as eosinophils, mast cells, and alveolar macrophages following immunologic stimuli (Samuelsson, 1983). The leukotrienes are potent mediators that can elicit many of the pathophysiologic features found in asthma and arthritis. Examples are smooth airway muscle contraction, microvascular leakage, and chemotaxis of inflammatory cells (Drazen and Austen, 1987). ABT-077 (zileuton), *N*-(1-(benzo[b]-thein-2-yl)ethyl)-*N*-hydroxyurea, is an orally active 5-lipoxygenase inhibitor discov-

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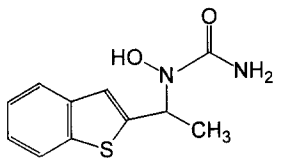

Fig. 1. Chemical structure of ABT-077 (Abbott-64077).

ered at Abbott Laboratories (see Fig. 1). This compound has been shown to inhibit the production of leukotrienes thus making it a relevant agent for alleviating airway muscular contraction in asthma (Hui et al., 1991; Bell et al., 1993). Intravenous as well as oral pretreatments of animals with ABT-077 have been found to be effective in inhibiting antigen-induced bronchoconstriction (data on file, Abbott Laboratories). An NDA has been filed for marketing an oral tablet formulation of this compound under the tradename Lotrin® in the United States and Leutrol® in Europe.

ABT-077 is rapidly cleared from the lungs after intra-tracheal instillation. Usually, within 2 h after dosing, the drug levels decrease to lower than those required for effective inhibition of bronchoconstriction. Over the years, liposomes have been clinically investigated for parenteral delivery of a variety of compounds (Gupta, 1990). More recently, liposomes have been investigated for controlled delivery of numerous therapeutic agents to the lungs (Gupta and Hickey, 1991). Examples include cytosine arabinoside (Juliano and McCullough, 1980), superoxide dismutase (Padmanabhan et al., 1985), pentamidine (Debs et al., 1987), benzylpenicillin (Mihalko et al., 1988), enviroxime (Gilbert et al., 1988), oxytocin (Mihalko et al., 1988), metaproterenol (McCalden et al., 1989), corticosteroids (Forsgren et al., 1990), cyclosporine (Gilbert et al., 1993) and insulin (Liu et al., 1993). One clinical study demonstrated more than a 30-fold increase in the mean residence time of sodium cromoglycate following nebulization to lungs as a liposome formulation as compared with the free drug (Kellaway et al., 1988). In view of these promising results, it was hypothesized that liposomal encapsulation might increase the in vivo residence time of ABT-077 following inhalation delivery (for asthma) or intra-articular administration (for arthritis). This report summarizes the results of in vitro studies conducted to evaluate the feasibility of liposomal encapsulation of ABT-077. First, 14 lipid compositions were screened for encapsulation of ABT-077 (see Table 1). The selected compositions were then optimized using a 3^3 factorial study.

Table 1
Lipid combinations considered for the preparation of ABT-077 liposomes

Batch #	Lipid composition[a]	Selection criteria	Reference(s)
1	DPPC:CHL:PA = 7:2:1	Clinically tested	Gregordias et al. (1974)
2	Egg PC:CHL:PA = 7:2:1	Clinically tested	Segal et al. (1976); Richardson et al. (1979)
3	Egg PC:CHL:PA = 9:9:1	Clinically tested	Begent et al. (1982); Barratt et al. (1984)
4	Egg PC:CHL = 1:1	Clinically tested	Eichler et al. (1988)
5	Egg PC:CHL = 4:1	Clinically tested	Osborne et al. (1983)
6	DMPC:DMPG = 7:3	Clinically tested	Lopez-Berestein et al. (1984); Perez-Solez et al. (1985)
7	DMPC:DMPG:CHL = 7:3:3	Tested at Abbott	
8	Egg PC:CHL:SA = 4:3:1	Clinically tested	Sculier et al. (1989)
9	POPC:PS = 7:3 PPC	Clinically tested	Murray and Kleinerman (1989)
10	DSPC:CHL:DCP = 12:7:3	Tested at Abbott	
11	DPPC:DPPG = 9:1	Clinically tested	Ivey et al. (1977)
12	DPPC:CHL = 1:1	Clinically tested	Kellaway et al. (1988)
13	DMPC:CHL = 3:1	Tested at Abbott	
14	DPPC:CHL:DPPA = 7:2:1	Clinically tested	McKeran et al. (1985); DeSilva et al. (1979)

[a] See Section 2.1. for the explanation of abbreviations.

2. Experimental

2.1. Materials

ABT-077 (zileuton) was manufactured by the Chemical and Agricultural Products Division of Abbott Laboratories. The following lipids were purchased from Avanti-Polar Lipids: egg phosphatidylcholine (PC), dipalmitoyl phosphatidylcholine (DPPC), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), 1-palmitoyl-2-oleolyl-sn-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-sn-glycero-3-phospho-1-serine (PS), distearoyl phosphatidylcholine (DSPC), 1-a-dipalmitoyl-phosphatidylglycerol (DPPG), and dipalmitoyl phosphatidic acid (DPPA). Some lipids were obtained from Sigma (cholesterol (CHL), phosphatidic acid (PA), dicetyl phosphate (DCP)) or Aldrich Chemicals (stearylamine (SA)). Ultra-spin polysulfone membrane cartridges, MW 100 000, were procured from Alltech and polyester 0.4 μm, 25 mm diameter membrane filters obtained from Nucleopore Corporation Filtration Products. Analytical grade reagents were purchased from Baker, Fisher, EM Science or Apper Alcohol and Chemical Co.

2.2. Equipment

A Waters HPLC pump, model 5907, a Waters 712 WISP automatic sample injector, a Kratos Spectroflow 783 programmable absorbance detector and a Spectraphysics SP4270 data recorder and integrator were used for the analytical work. A NICOMP Particle Sizing System, Autodilute Model 370, was used to measure the particle size of different preparations.

2.3. Formulation screening

Stock solutions of different lipids were prepared in absolute alcohol at concentrations of 50–100 mg/ml, using gentle heating ($\sim$ 50°C) as necessary (e.g. CHL) for complete dissolution. An ethanolic stock solution of drug, at a concentration of 2 mg/ml, was also prepared. Appropriate volumes of lipid solutions (0.05–1.0 ml) were added to a tube according to a given liposomal preparation such that it contained 100 mg of total lipids. Thereafter, 0.5 ml of drug solution was added to that tube to give a drug: lipid ratio of 1:100 (dry weight basis). The tubes were vortexed for approximately 2 min and then placed in Driblock DB-3® at 40–50°C and purged with air to facilitate the evaporation of ethanol. After 8–24 h of evaporation, the resulting lipid/drug film was hydrated by adding 10 ml of 0.05 M phosphate buffer, pH 6.5. The tubes were briefly vortexed and/or sonicated ($\leq$ 5 min) and then allowed to shake at 60–70 rpm at RT for 2 h. Thereafter, the preparations were filtered via 0.8 μm Nalgene® disposable filters into 10 ml sterile glass bottles. All formulations were stored at 4°C and characterized within one week of preparation.

2.4. Formulation optimization

A 3^3 factorial study was conducted to investigate the effect of lipid composition (9–50% w/w CHL in PC), drug level (1–25% w/w of total lipid) and extent of formulation dilution (0, 1:25 and 1:100) on drug loading and drug encapsulation in liposomes. Ethanolic solutions of CHL and PC were prepared at concentrations of 50 mg/ml. In addition, ethanolic solutions of ABT-077 were prepared at concentrations of 5, 32.5 and 41.67 mg/ml. Appropriate volumes of each lipid solution (0.18–1.82 ml) were added to a tube such that it contained 100 mg total lipids. Thereafter, 0.2–0.6 ml of drug solution, of appropriate concentration, was added to that tube. The total volume of lipid and drug solution per tube varied between 2.0 and 2.4 ml. On a dry weight basis, the drug: lipid ratio ranged between 1:100 and 1:4. Thereafter, the tubes were processed in a manner similar to that described above. Small aliquots of each liposome formulation were diluted 0-, 25- and 100-fold, allowed to shake at 50–60 rpm at RT for 30 min, and then filtered through a polysulfone membrane cartridge to determine the effect of dilution on drug loading. All formulations were stored at 4°C and characterized within one week of preparation. All experiments were conducted in triplicate.

2.5. Analytical methodology

A reverse-phase HPLC method was employed for drug analysis. The mobile phase composition was 70:15:10:5 v/v aqueous buffer:acetonitrile: tetrahydrofuran:methanol. The aqueous buffer consisted of 40 mM sodium phosphate, 7.5 mM phosphoric acid and 5.0 mM acetohydroxamic acid. A 250 × 4.6 mm i.d. 5 μm Supercosil C-18 column was used. The detection wavelength was set to 260 nm. Typical injection volumes were 50 μl, the mobile phase flow rate was 1 ml/min and absorbance range used was 0.01 AUFS. Under these conditions, the run time per injection was 30 min and the retention time for ABT-077 approximated 20 min. The drug concentration of unknown samples was determined using least square regression analysis of standards dispersed throughout the samples.

2.6. Formulation characterization

2.6.1. Particle shape and size

Selected samples were subjected to negative stain scanning electron microscopic (SEM) observation for visualization of the structure of the liposomes. A NICOMP submicron particle sizer was used to measure the particle size and size distribution of various preparations.

2.6.2. Lipid versus aqueous buffer distribution coefficient of ABT-077

Drug concentrations in lipid versus aqueous media (0.05 M phosphate buffer, pH 6.5) were used to determine the distribution coefficient of drug. ABT-077 concentrations in the liposome suspending medium were determined chromatographically after isolating the lipid-free fractions, as follows: A sterile ultra-spin centrifuge filter, consisting of a 100 000 MW cut-off polysulfone ultrafiltration membrane cartridge, was used to isolate clear aqueous supernatant from the liposome preparations. Physical as well as chemical compatibility of the filter cartridge was ensured by filtering a 100 μg/ml drug solution through it, wherein the drug recovery was quantitative. About 0.5 ml of each formulation was transferred to filter cartridges housed in a TDX centrifuge tube. The tubes were spun at 10 000 rpm for 5 min to obtain clear filtrates which were analyzed for drug concentration using HPLC. The difference of drug added per tube and that determined in the aqueous medium was used to represent drug partitioned into the lipid phase.

2.6.3. Drug leaching from liposomes

The effect of various lipid compositions on leached drug was assessed by washing liposome pellets with 0.05 M phosphate buffer, pH 6.5, and analyzing the washings for drug concentration. After filtering 0.5 ml of each liposome preparation, as described above, 0.5 ml of fresh 0.05 M phosphate buffer pH 6.5 was added to the filter cartridge containing the lipid pellet. The cartridge was then placed in TDX centrifuge and spun at 10 000 rpm for 5 min to obtain a clear washing which was called the first wash. The process was repeated once more with the lipid pellet obtained after the first washing to collect the second wash. Both washings were analyzed for drug concentrations by HPLC.

2.6.4. Drug loading and drug encapsulation efficiency in liposomes

Drug concentration in aqueous fractions of the liposomes were used to estimate drug loading and drug encapsulation efficiency in the lipid phase. The amount of drug associated with the lipids, estimated as the difference of drug initially added and that monitored in the aqueous fraction, was expressed as drug loading in units of μg ABT-077 per mg lipid.

The encapsulation efficiency of drug in the liposomes was expressed as:

$$\frac{\mu g \text{ drug/mg lipid (assayed)}}{\mu g \text{ drug/mg lipid (theory)}} \times 100 \quad (1)$$

2.7. Statistical treatment

Three factor analysis of variance on the data was performed using Statgraphics® (Statistical Graphics Corporation).

3. Results and discussion

3.1. Rationale for lipid selection

The appropriate selection of lipids has been known to allow preparation of liposomes with specific properties, e.g. desired size, charge, preferential in vivo distribution, stability and toxicity. One of the key criteria for lipids selection in this investigation was their relatively low in vivo toxicity. Fourteen lipid compositions were selected for initial screening study based on their safety profile. These lipid compositions are described in Table 1.

3.2. Formulation screening studies

3.2.1. Preparation of liposomes

All liposome formulations were prepared by solvent evaporation from the lipid/drug mixture followed by hydration in aqueous buffer. An empirical drug: lipid ratio of 1:100 was used. The resulting drug and lipid concentrations in the final suspension were 0.1 and 10 mg/ml, respectively. The aqueous solubility of ABT-077 is approximately 0.17 mg/ml (Alvarez and Slade, 1992). Hence, if the drug was to preferentially reside in the aqueous media, the theoretical drug concentration would be less than its saturation solubility. ABT-077 is most stable in a pH range of 3.0–7.0 (Alvarez and Slade, 1992). Hence, an aqueous buffer, pH 6.5, was used for hydrating and suspending the lipid/drug film. Overall, these experiments were anticipated to allow a good assessment of the possibility of drug encapsulation in liposomal systems.

All lipid combinations were easily processed to obtain a lipid/drug film, with ethanol evaporation time ranging between 8 and 12 h. Following hydration with aqueous buffer, all formulations were easily filtered via 0.8 μm Nalgene® disposable filter. The only exception was batch no. 11 which did not suspend well. Hence, testings on this batch were discontinued.

The mean number diameter of most preparations ranged between 100 and 250 nm. A representative negative stain photomicrograph of a liposomal preparation of ABT-077 (batch no. 4) is shown in Fig. 2. These data suggest the liposomes were probably multilamellar in structure. This is consistent with the preparation method used above, following passage through a 0.8 μm filter.

3.2.2. Lipid versus aqueous buffer distribution coefficient of ABT-077

The lipid versus aqueous buffer distribution coefficient of ABT-077, K, was estimated as a ratio of drug concentration in lipid layers (C_l) versus drug concentration in aqueous medium (C_{aq}), i.e.

$$K = C_l / C_{aq}$$

Due to differences in the lipid and aqueous phase volumes, the above relationship was rearranged to

$$K = [(\text{Amount of drug in lipid})/(\text{Weight of lipid})] \times [(\text{Amount of drug in aqueous medium}/(\text{Weight of aqueous medium})]^{-1} \quad (2)$$

The amount of drug in aqueous medium was determined by analyzing liposomal supernatant fraction, after filtration through a polysulphone ultra-filtration cartridge, and assuming a density of 1 g/ml. The amount of drug in the lipid fraction was estimated by mass-balance, i.e. difference of the amount of drug added per formulation and that determined in the respective liposomal supernatant. These calculations assume no drug loss during the formulation processing.

Table 2 summarizes drug concentrations in supernatant fraction of various liposomal preparations. With a control sample, without any lipid, the ABT-077 concentration was found to be 99 μg/ml. The difference between 99 μg/ml (maximum possible concentration of ABT-077 in the aqueous medium) and that actually determined in the supernatant fraction of various liposome batches reflects the fraction of drug partitioned into and/or associated with the lipids and hence possibly available for modulated release. Table 2 also summarizes K and $\log K$ data for various lipid compositions. The $\log K$ values ranged between 1.4 and 2.5, the latter being obtained with 7:3 POPC:PS, i.e. batch no. 9. The next highest

Fig. 2. A negative stain photomicrograph of a representative batch of ABT-077 liposomes.

log K, ≥ 2.1, was obtained with PC:CHL combinations (batches 4 and 5). Further studies are required to correlate the effect of lipid properties on partitioning of ABT-077 during liposome formation.

3.2.3. Drug loading and leaching studies

The effect of various lipid compositions on leached drug was assessed by washing liposome pellet with aqueous buffer, twice, and analyzing both washings for drug concentration by HPLC (see Table 2). However, with batches # 8 and 9, neither lipids could be pelleted nor clear filtrate be obtained even after several prolonged centrifugation cycles. Hence these batches could not be tested for potential drug leaching.

The effect of various lipid combinations on drug loading is summarized in Table 3. The maximum theoretical drug loading was 10 μg/mg lipid. Some liposome preparations resulted in low drug loadings, i.e. ≤ 2 μg/mg (e.g. batches # 10 and 14) and others yielded drug loadings ≥ 6 μg/mg (e.g. batches # 5 and 9).

The determination of drug concentration in liposomal washings allowed assessment of drug loading in washed samples. This provided an opportunity for the selection of lipid combinations with high drug loadings before and after two washings. With most preparations, 20–50% of the initial drug load was lost during the first wash, and a total of 35–65% was lost in the two washes (see Table 3). The 7:3 POPC:PS lipid combination, batch # 9, which yielded highest log K and drug loading, lost only 25% of the initial drug load during the first washing. However due to difficulty in homogeneously suspending this lipid, a second washing was not performed. Hence, its ability to retain ABT-077 during subsequent washings could not be assessed. The 1:1 and 4:1 PC:CHL combinations (batches 4 and 5, respectively), which yielded the next highest drug loadings (i.e. 5.6 and 6.3 μg/mg, respectively), also lost

Table 2
Summary of ABT-077 concentration and distribution coefficients in various liposomal preparations

Batch #	Drug concentration (μg/ml)			Distribution coefficient	
	Supernatant	1st wash	2nd wash	K^f	Log K^f
Control (no lipids)	99.0				
1	72.32	15.32	5.41	38.27	1.58
2	54.16	17.90	6.75	84.64	1.93
3	61.03	17.13	6.54	63.85	1.81
4	43.71	16.81	7.05	128.78	2.11
5	37.31	16.10	9.49	168.02	2.23
6	63.62	11.04	6.17	57.18	1.76
7	48.21	20.42	12.28	107.43	2.03
8	72.09[a]	—[b]	—[b]	38.72	1.59
9	23.72	18.16	—[c]	321.59	2.51
10	79.97	7.90	3.92	25.05	1.40
11	—[d]	—[d]	—[d]	—[e]	[e]
12	72.71	11.60	4.04	37.53	1.57
13	52.38	16.42	9.46	90.91	1.96
14	81.52	10.24	2.45	22.67	1.36

[a] Supernatant not very clear.
[b] Did not perform washings on this sample.
[c] Lipid particles floated on the surface of suspending medium making it difficult to isolate clear sample.
[d] Liposomes could not be made due to difficulty in hydrating and suspending lipid/drug film.
[e] Since liposomes in this batch could not be made, K and log K values are not determined.
[f] Obtained using data from unwashed samples.

20–25% drug during the first wash. The total loss in their drug load, after two washings, approximated 35%. In view of good clinical safety results with these lipids (Eichler et al., 1988; Osborne et al., 1983), this lipid combination was selected for the optimization study.

3.3. Formulation optimization

3.3.1. Preparation of liposomes

Table 4 shows the protocol for the 3^3 factorial optimization study. Nine different liposomal formulations of ABT-077 containing 9–50% w/w CHL in PC and 1–25% w/w drug were prepared.

Since some formulations contained drug quantities greater than the aqueous saturation solubility of ABT-077 (e.g. batches 4 through 9 in Table 5), the lipid versus aqueous phase distribution coefficient estimates with these samples could be erroneous. Theoretically, the dilution of these preparations with buffer to levels such that the aqueous drug concentration is significantly less than its saturation solubility should favor transfer of drug to the aqueous phase. For example, with preparations containing 25 mg ABT-077/100 mg lipids, the undiluted, 25- and 100-fold diluted samples contained 2500, 100 and 25 μg/ml drug, respectively. Since the aqueous solubility of ABT-077 is 170 μg/ml (Alvarez and Slade, 1992), the dilution steps were anticipated to provide a more realistic information on drug distribution coefficient, leaching, and hence loading. The dilution step also mimics sink conditions encountered following in vivo administration. With this rationale, small aliquots of all formulations were diluted 25- and 100-fold.

3.3.2. Drug distribution coefficient, loading and encapsulation

The apparent distribution coefficient of drug in the original formulations, and 25- and 100-fold diluted samples, was assessed using Eq. (2). In general, the log K values ranged between 2.2 and 3.4. The drug loading and percent drug encapsu-

Table 3
Drug loading in various liposomal preparations

Batch #	Drug loading (μg ABT-077/mg lipid)		
	Fresh liposomes[a]	After 1st wash[b]	After 2nd wash[b]
1	2.77	1.24 (44.76)[c]	0.70 (25.27)[c]
2	4.58	2.79 (60.92)	2.12 (46.29)
3	3.90	2.19 (56.15)	1.54 (39.49)
4	5.63	4.50 (79.92)	3.80 (67.50)
5	6.27	4.66 (74.32)	3.71 (59.17)
6	3.64	2.54 (69.78)	1.92 (52.74)
7	5.18	3.14 (60.62)	1.91 (36.87)
8	2.79	—[d]	—[d]
9	7.63	5.81 (76.15)	—[e]
10	2.00	1.21 (60.50)	0.82 (41.00)
11	—[e]	—[e]	—[e]
12	2.73	1.57 (57.51)	1.17 (42.86)
13	4.76	3.12 (65.55)	2.17 (45.59)
14	1.85	0.83 (44.86)	0.59 (31.89)

[a] Determined as [(Amount of drug initally added)−(Amount of drug determined in supernatant)]/(Total amount of lipid used in the formulation).
[b] Determined as [(Amount of lipid in wash sample × Its drug load)-(Amount of drug released)]/(Amount of lipid in washing sample).
[c] The figure in parentheses refers to percentage of initial drug loading.
[d] Supernatant not very clear, hence did not perform washings on this sample.
[e] Lipid particles floated on the surface of suspending medium making it difficult to isolate clear sample.

lated data for different preparations is summarized in Table 5. For the undiluted samples, 6–230 μg/mg drug loading was achieved. Twenty five-fold dilution of the samples had a little effect on drug loading of samples prepared with 1 mg drug/100 mg lipid (e.g. batches 1 through 3 in Table 5); however for samples containing medium and high levels of drug (i.e. 13 and 25 mg/100 mg lipid, respectively), the drug loading was reduced by 50–75%. One hundred-fold dilution caused further reduction in drug loading; nonetheless, the samples prepared with medium and high drug levels allowed drug loadings of 20–50 μg/mg (see Table 5). These data suggest that the dilution of samples causes the transfer of drug from the non-aqueous to the aqueous phase. However, it is encouraging to note that despite dilution to the extent that theoretical drug concentrations were significantly lower than the saturation solubility of ABT-077, reasonable liposomal drug loadings were achieved. One implication of this finding is that if these liposomes were diluted after in vivo administration, an appreciable fraction of drug would remain associated with the liposomes.

Table 4
A 3^3 factorial study template used for the optimization of ABT-077 liposomal preparations

Factors	Levels		
	Low	Medium	High
Drug level (mg/100 mg lipids)	1.0	13.0	25.0
Cholesterol content (% w/w of total lipids)	9.0	35.0	50.0
Dilution of formulation	0	1:25	1:100

Approximately 65–95% of the total drug was encapsulated in the undiluted samples (see Table 5). However with dilution, it reduced to approximately 15–20%. This data suggests that the drug fraction which is retained after 100-fold dilution may be available for modulated release in vivo. In view of the complexity of assessing liposomal drug release characteristics in vitro, animal studies are required to test the hypothesis of modulated drug release in vivo.

The analysis of variance indicated that lipid composition as well as the combined effects of drug level and dilution significantly influence $\log K$ ($p \leq 0.0256$ and $p \leq 0.00005$, respectively). However, the two effects were independent of each other. These factors were also found to significantly effect drug loading in liposomes ($p \leq 0.0222$ and $p \leq 0.00005$, respectively). Fig. 3A displays the effect of lipid composition on $\log K$ values. The data in this figure represents an average of three values derived at different drug loadings plus three values derived at different dilution levels, i.e. each data point represents an average of $3^3 = 9$ determinations. As can be seen, the increase in cholesterol content was found to linearly increase $\log K$ ($r^2 = 0.994$). Fig. 3B shows the effect of cholesterol content on drug encapsulation in liposomes ($r^2 = 0.997$) and suggests that cholesterol improves the encapsulation of ABT-

Table 5
Mean (± SD) drug loading and percent of drug encapsulated in liposomal preparations tested as a part of 3^3-factorial study[b]

Batch #	Composition		Undiluted samples		1:25 diluted samples		1:100 diluted samples	
	% w/w CHL in Egg PC	Drug (mg/100 mg lipids)	Drug loading (μg drug/mg lipid)	% drug encapsulated	Drug loading (μg drug/mg lipid)	% drug encapsulated	Drug loading (μg drug/mg lipid)	% drug encapsulated
1	9	1	8.77 ± 0.33	87.70 ± 3.27	4.10 ± 1.63	41.0 ± 16.26	1.38 ± 0.58	13.80 ± 5.80
2	35	1	7.09[a]	70.92[a]	4.95[a]	49.50[a]	0.54[a]	5.40[a]
3	50	1	6.44 ± 0.17	64.39 ± 1.67	5.04 ± 2.11	50.38 ± 21.04	0.54 ± 0.50	5.40 ± 7.07
4	9	13	117.99 ± 0.21	90.76 ± 0.18	34.17 ± 4.01	26.28 ± 3.08	26.85 ± 3.32	20.66 ± 2.55
5	35	13	116.32 ± 0.83	89.47 ± 0.64	29.64 ± 8.50	22.80 ± 6.54	22.50 ± 3.39	17.31 ± 2.61
6	50	13	117.26 ± 0.06	90.20 ± 0.06	20.17 ± 4.55	15.51 ± 3.49	18.65 ± 1.34	14.35 ± 1.03
7	9	25	235.77 ± 0.98	94.31 ± 0.39	91.47 ± 13.13	36.59 ± 5.25	45.20 ± 13.15	18.08 ± 5.26
8	35	25	234.16 ± 0.71	93.66 ± 0.28	102.34 ± 10.24	40.94 ± 4.09	46.60 ± 1.84	18.64 ± 0.74
9	50	25	235.96 ± 0.08	94.39 ± 0.04	62.99 ± 18.05	25.20 ± 7.22	40.20 ± 7.07	16.08 ± 2.83

[a] Only one experiment in this set.
[b] Drug loading referes to amount of drug entrapped per unit weight of lipids. The % ABT-077 encapsulated referes to percentage of total added drug which is associated or entrapped, and is presumally available for controlled drug delivery.

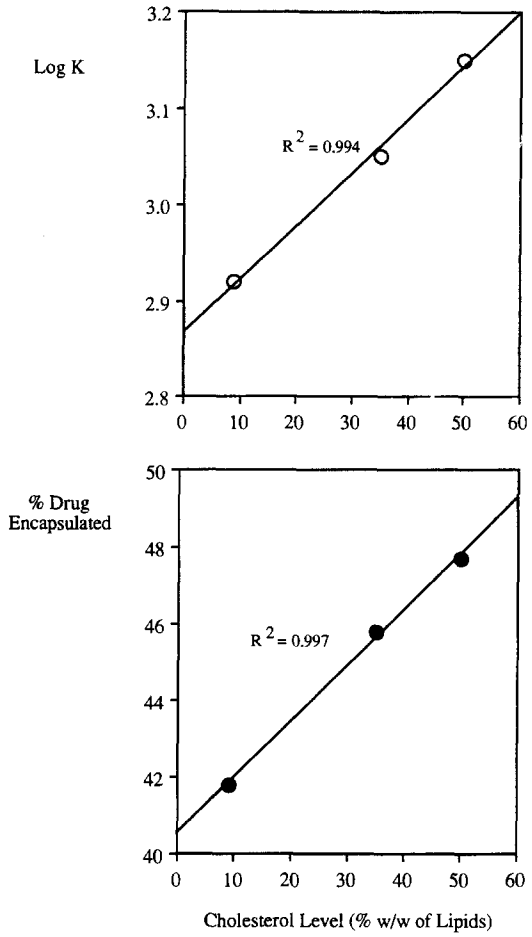

Fig. 3. Effect of cholesterol level on log K (○) and drug encapsulation in liposomes (●). Note that the Y-axis represents data averaged for the drug level and extent of formulation dilution.

077. These results are encouraging because cholesterol has been reported to improve the stability of liposomes containing egg PC (Gonzalez-Rothi et al., 1991).

4. Conclusions

This study has shown that encapsulation of ABT-077 in liposomes is feasible. However, animal studies are needed to assess the improvement in the residence time of drug in the lungs. Such studies may also provide information regarding the need for further optimization of these preparations.

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