TABLE 2: SPARFLOXACIN KINETICS WITH AND WITHOUT ANTACID TREATMENT IN HEALTHY VOLUNTEERS

Pharmacokinetic Parameters	Sparfloxacin	Sparfloxacin + Antacid
C_{max} (μg/ml)	0.87±0.85	0.34±0.158
T_{max} (h)	4.00±0.07	4.00±0.07
K_{El} (h^{-1})	0.04±0.04	0.06±0.04
$t_{1/2el}$ (h)	15.7±0.57	12.0±1.12
$AUC_{36-\infty}$ (μg/ml.h)	8.84±4.03	3.67±2.12

Values represent mean±SEM, n=6; P<0.05 when compared to sparfloxacin alone.

the standard and the diameter of the zone of inhibition.

The mean zones of inhibition obtained with plasma are presented in Table 1. Results showed that the zones of inhibition increased with increasing concentrations of sparfloxacin over the range tested. The mean plasma concentration of sparfloxacin with antacid was lower as compared to sparfloxacin alone. The difference was statistically significant at 1, 2, 3, 4, 5, 6, 24 and 36 h (P<0.05). Table 2 compares various pharmockinetic parameters (mean±SEM) of sparfloxacin before and after single oral dose of antacid. C_{max} and $AUC_{36-}\alpha$ of sparfloxacin with antacid were significantly, lower as compared to sparfloxacin alone (P<0.05)

When antacid was administered along with sparfloxacin the C_{max} and $AUC_{36-}\alpha$ of sparfloxacin has been significantly lowered. The bioavailability of sparfloxacin is reduced due to the presence of aluminum and magnesium ions.

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Effect of Cholesterol on Size Distribution of Freeze-thaw Extruded Liposomes

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Accepted 25 May 2004

Revised 10 February 2004

Received 1 September 2003

The effect of cholesterol on vesicle size during freezing and thawing process of extruded egg phosphatidylcholine liposomes were studied by varying the length and number of freeze-thaw cycles. Laser diffraction particle size analysis showed that the volume median diameter of freeze-thawed egg phosphatidylcholine multilamellar vesicles was increased when cholesterol was included in the bilayers. Using a freeze-thaw cycle of 3 min freezing in liquid nitrogen at –196° followed by 3 min thawing at 50° resulted in an anomalously large particle size for egg phosphatidylcholine/cholesterol formulations. When egg phosphatidylcholine/cholesterol multilamellar

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vesicles were repeatedly freeze-thawed, the maximum size was achieved after five freeze-thaw cycles.

Liposomes are microscopic vesicles consisting of membrane like lipid bilayers surrounding an aqueous media. The lipid vesicles are formed spontaneously when phospholipids are hydrated in aqueous media[1]. Most of the liposomal preparation methods involve freeze-thaw and extrusion steps. Repeated freezing and thawing serves to break apart the closely spaced lamellae of the vesicles thereby raising the trapping efficiency by increasing the ratio of aqueous solute to lipid[2]. Moreover, extrusion of frozen and thawed multilamellar vesicles (MLVs) results in production of unilamellar liposomes more readily than those made by conventional techniques[3]. The frozen and thawed extruded liposomes were a monodisperse population with an internal volume higher than large unilamellar vesicles prepared solely by extrusion of MLVs through polycarbonate filters of equivalent pore size[4].

Most of the potential factors affecting the liposomes during freezing and subsequent thawing are directly related to behavior of water in the dispersion[5], cooling rate[6], phospholipid concentration[7] and localization of any additives[8]. Invariably, cholesterol is an integral part of any liposomal formulations and is likely to influence the vesicle properties of liposomes prepared by freeze-thaw and extrusion method. The purpose of this study was to investigate the effect of cholesterol on vesicular size by varying the length and number of freeze-thaw cycles.

Egg phosphatidylcholine (eggPC) was purchased from Lipoid, Milan, Italy and used as received. Cholesterol (99%+ purity) was obtained from Sigma Chemicals, St. Louis, MO, USA and chloroform from E. Merck Ltd., Mumbai. All other reagents used were of analytical grade.

Liposomes of eggPC and eggPC/chol (1:1 mol ratio) were prepared by lipid layer hydration method. Briefly, weighed quantity of EggPC with or without cholesterol was taken in a round bottom flask and chloroform was added to dissolve the lipids. The organic solvent was removed by rotary evaporation under vacuum, in a water bath at 55° for 15 min. The flask was then flushed with nitrogen for 2 min to remove traces of residual solvent. An appropriate volume of filtered, double distilled, deionised water was added to the dry film in the flask to give a final phospholipid concentration of 15 mg/ml. Glass beads are added to aid dispersion, the flask flushed with nitrogen, gently rotated for 45 min in the water bath, and shaken to produce MLVs. The suspension was annealed for a further 2 h in the water bath before storage under nitrogen in a refrigerator at 4°.

EggPC and EggPC/chol MLVs were extruded 10 times through a 2 μm polycarbonate filters (Sartorius GmbH, Goettinger, Germany), held in a 25 mm syringe filter holder. Vesicles were sized on a Malvern droplet and particle sizer (Series 2600C, Malvern Instruments, Worcestershire, UK). The instrument's software expresses particle size as volume median diameter (VMD). Sizing data are presented as the mean±S.D. of three independent analyses. Aliquots from each dispersion (4 ml) were taken in 20 ml liquid scintillation vials (FBG-Trident, London, UK) and quench frozen in liquid nitrogen at −196° for the specified duration (1-5 min). The vials were transferred immediately to a water bath and held at 50° for an equal duration. The freezing and thawing process was repeated for a specified numbers of cycles. This was kept at room temperature in sealed container for 1 h before size analysis by laser diffraction. All experiments were performed in triplicate.

The mean VMD of eggPC MLVs was not altered by freeze-thawing and the duration of each freeze-thaw cycle had no clear effect on the mean VMD of eggPC vesicles (fig. 1). The mean VMD of all eggPC/chol MLVs significantly increased following repeated freeze-thawing (fig. 1), with the greatest size increase observed for samples subjected to ten '3-min' freeze-thaw cycle. The analysis was repeated for an additional three times at this data point (i.e. n=6). The observed increase in size was reproducible, suggesting that the presence of cholesterol resulted in the formation of large liposomes or aggregates during freeze-thawing, particularly with a 3-min cycle. Cholesterol modifies the physical structure of phospholipid bilayers, restricting bilayer permeability and the movement of hydrocarbon chains at temperature above the phospholipid main transition[9]. The inclusion of cholesterol might have reduced the water permeability and compressibility of liposome bilayers[10], decreasing their ability to withstand internal expansion due to ice formation during fast freezing, and causing bilayers to rupture during freeze-thawing with new liposomes forming as bilayer fragments re-assemble. In addition, cholesterol has been reported to induce aggregation and fusion of freeze-thawing of DPPC liposomes[11]. The observed increase in mean size for eggPC MLVs when cholesterol was included indicates that larger structures were formed, which may have been

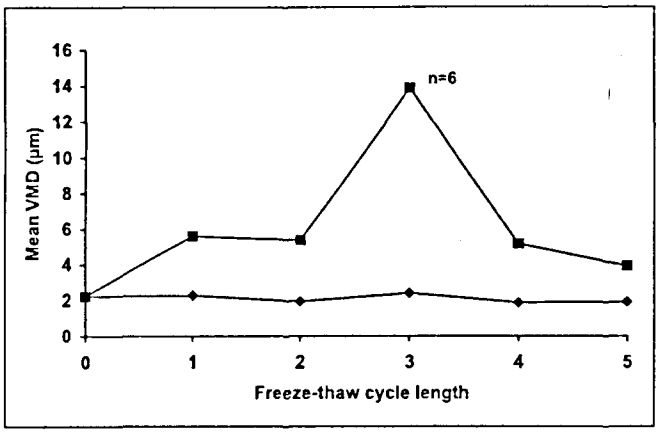

Fig. 1: Effect of cholesterol on vesicle size by changing freeze-thaw cycle length.

Influence of cholesterol by changing the freeze-thaw cycle length on the mean VMD of MLVs extruded 10 times through a 2 µm pore filter then freeze-thawed 10 times. (-♦-) eggPC; (-■-) eggPC/chol (1:1). Freeze thaw cycle length is the number of minutes preparation was held at -196° and subsequently at 50°. (n=3±S.D.).

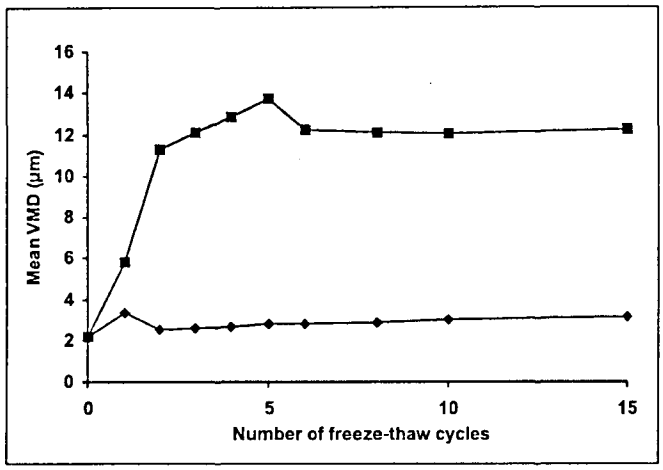

Fig. 2: Effect of cholesterol on vesicle size by changing the number of freeze-thaw cycles.

Influence of cholesterol by changing the number of '3-min' freeze-thaw cycles on the mean VMD of MLVs (previously extruded 10 times through a 2 µm pore filter). (-♦-) eggPC; (-■-) eggPC/chol (1:1). (n=3±S.D.).

due to aggregation and subsequent fusion of vesicles.

It was observed macroscopically, that samples exposed to 3-min freeze-thaw cycles did melt completely during each thaw stage unlike 1- and 2-min cycles. Consequently, this preparation may not have been warmed completely, with incomplete mixing, possible resulting in insufficient time for disrupted bilayers to re-anneal. Therefore liposome fragments may have suffered further freeze-thaw damage before re-assembling. Vesicles exposed to 4- and 5-min freeze-thaw cycles had more time to re-assemble during each thaw stage and this may have been reflected by smaller increase in mean diameter.

The mean VMDs of eggPC MLVs increased after one freeze-thawing cycle, but did not change appreciably between one and 15 cycles (fig. 2). However, the mean VMD of eggPC/chol MLVs increased following the process up to five cycles (fig. 2). Subsequent increase in the number of cycles produced no significant change in vesicle diameter, indicating an equilibrium situation after which further freeze-thaw cycles had little or no effect on the mean diameter for these liposomes. Thus the increased rigidity of eggPC bilayers due to inclusion of cholesterol seemed to have a destabilizing effect during freeze-thawing, causing the liposomes to rupture and subsequently re-assemble to form a new population of liposomes with a higher mean VMD and wider and more variable size distribution.

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