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Liposome Encapsulated Rifampicin and Isoniazid

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Rifampicin and isoniazid (INH) in combination were formulated into liposomes for utilization as a delivery system for optimum therapeutic use in tuberculosis. A modified lipid layer hydration method was employed to prepare liposomes. The formulated liposomes were characterized for size distribution, drug loading and *in vitro* drug release studies. The liposomes prepared were in the size range 3.3 to 26.4 mm the mean diameter being 8.64mm. Entrapment efficiencies of 32.06% and 72.42% were obtained for INH and rifampicin in the liposomes respectively. *In vitro* release study on liposomes indicated 53.42% release for isoniazid and 35.99% for rifampicin. Stability study was carried out at different temperatures and it was found to be stable. *In vivo* antibacterial activity was determined by agar plate method in albino rats following the administration of liposomal formulation in comparison with drug alone and control. The liposomal drugs exhibited larger zone of inhibition as compared to the free drugs. Thus, it is evident from this study that liposomes could be promising delivery systems for rifampicin and INH with prolonged drug release profiles and better therapeutic effect.

Tuberculosis (TB) is responsible for more deaths than any other infectious disease. It also shares a deadly synergism with HIV. As a result, countries with a higher prevalence of HIV are witnessing an epidemic of tuberculosis on a massive scale[1]. There have been no significant new drugs developed for tuberculosis for over 30 years, and it seems unlikely there will be any more for several years to come. Rifampicin and INH are the most effective drugs for the treatment of TB. Rifampicin, like INH should never be used alone for this disease, because of the rapidity with which resistance may develop. The combination of INH and rifampicin is probably as effective, for sensitive microorganism, as regimens that utilize three or more agents[2]. The behaviour of drugs *in vivo* can often be changed in a dramatic fashion by coupling the drug to a carrier moiety. The plasma clearance kinetics, tissue distribution, metabolism and cellular interaction of the drug will be dictated, or at least strongly influenced, by the behaviour of the carrier. Liposomes are well suited for improving the efficacy and potency of existing drugs by providing a means for entrapment and delivery to cells of variety of compounds, including highly ionized, high molecular weight or lipophilic drugs, protection of the drug from degradation in the blood stream, natural targeting to cells, belonging to monophagocytic system as well as specific targeting to other cells, sustained release of drug and buffering of drug toxicity[3,4].

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MATERIALS AND METHODS

Isoniazid and rifampicin were the gift samples from Cadila Health Care, Ahmedabad. Cholesterol, dicetyl phosphate, egg phosphatidyl choline and sigma dialysis sac were from Sigma Chemicals Co., St. Louis, USA. All other reagents were of analytical grade.

Preparation of liposomes:

Liposomes were prepared by adopting the procedure of Azmin *et al.*,[5] with slight modification. Accurately weighed quantity of rifampicin was dissolved in minimum quantity of chloroform taken in a round bottom flask. To

this cholesterol, dicetyl phosphate and egg phosphatidylcholine were added and dissolved in diethyl ether taken in a round bottom flask. The flask was rotated at 1.5 cm above a water bath at 40-50° under reduced pressure (10-15 mmHg) until all the organic phase evaporated and a slimy layer was formed on the wall of the round bottom flask (8-10 min). Ten millilitres of PBS containing isoniazid was added to the film with gentle agitation. The mixture was intermittently mixed on a vortex mixer to get a uniform suspension.

Size distribution analysis:

Size distribution of liposomes was evaluated by optical microscopy using a calibrated eyepiece micrometer. About 200 liposomes were measured individually, average was taken, and their size range and mean diameter were calculated.

Measurement of entrapment efficiency:

Separation of free drug was carried out using gel filtration using Sephadex G50. Liposomes, which eluted out first could be made out as slightly dense, white opalescent suspension. Collection was continued till no turbidity was seen. Elution was continued again, the unentrapped drug was collected to determine the extent of entrapment difference. Elution was taken complete when no difference in absorption could be detected between the eluent and normal saline. The absorbance of unentrapped drug was measured after suitable dilution. From the mass balance, the quantity of the entrapped drug in the liposomes was calculated. The experiment was done in triplicate.

***In vitro* drug release studies:**

Liposomes containing known amount of drug were subjected to *in vitro* release analysis in phosphate buffer saline (PBS 7.4) using Sigma dialysis sac. At different time intervals, 10 ml of samples were withdrawn and drug content was determined spectrophotometrically at 266 and 334 nm using the simultaneous estimation method. Unentrapped drug was determined spectrophotometrically.

Stability studies:

The formulated liposomes containing known amount of the drugs were divided into three portions. One portion was kept at room temperature (25°) and the second portion at 37° and the third at 4° for one month. After a 7-day interval, these were evaluated for their drug content Fig. 2,3.

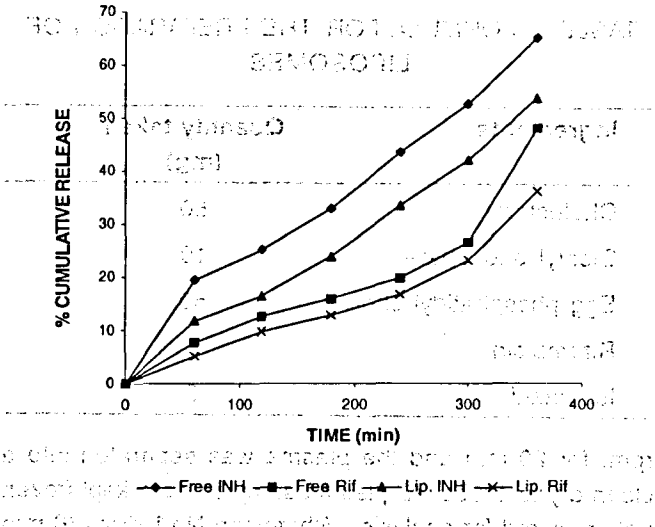

Fig 1: *In vitro* release studies from Rif-INH liposomes in PBS pH 7.4 in a sigma dialysis sac

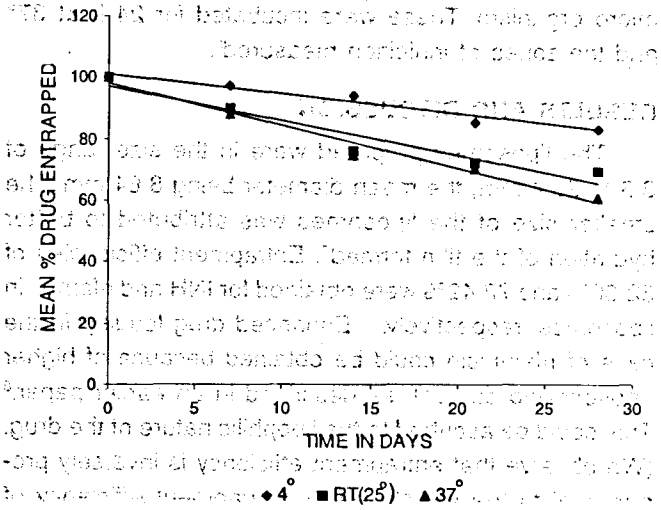

Fig 2: Stability studies of Rifampicin from Rif-INH liposomes

***In vivo* studies:**

Antibacterial study was conducted by disc diffusion method. The formulations were given intraperitonially to albino rats divided into 3 groups of 6 animals each. One group received 10 mg/kg body weight of rifampicin and INH in PBS (pH 7.4). For the second group 10 mg/kg body weight of rifampicin-INH liposomes were administered. The third group served as a control. From each rat, blood samples (2 ml) were collected into heparinised tubes at 0, 0.5, 2, 4, 6, 8, and 10 h from the sino-orbital vein. Minimum of 3 rats were used for each interval of blood collection. The blood samples were centrifuged at 3500

TABLE 1: FORMULA FOR THE PREPARATION OF LIPOSOMES

Ingredients	Quantity taken (mg)
Cholesterol	50
Dicetyl phosphate	10
Egg phosphatidyl choline	50
Rifampicin	9
Isoniazid	6

rpm. for 20 min and the plasma was separated into a clean dry test tube. All plasma samples were kept frozen until required for analysis. Whatmann No.1 discs (6 mm diameter) containing the above plasma samples were placed over the nutrient agar plate inoculated with the micro organism. These were incubated for 24 h at 37° and the zones of inhibition measured[6].

RESULTS AND DISCUSSION

The liposomes prepared were in the size range of 3.3 to 26.4 mm, the mean diameter being 8.64 mm. The smaller size of the liposomes was attributed to better hydration of the film formed[7]. Entrapment efficiencies of 32.06% and 72.42% were obtained for INH and rifampicin liposomes, respectively. Enhanced drug loading in the case of rifampicin could be obtained because of higher phospholipid content, as described in an earlier paper.[8] This could be ascribed to the lipophilic nature of the drug. (We observe that entrapment efficiency is inversely proportional to the vesicle size[3]). Entrapment efficiency of 79.2% was reported for liposomes containing only rifampicin[6].

In vitro release was performed using sigma dialysis sacs. It was observed that liposomes could release up to 53.4% of INH and 35.99% of rifampicin. In case of free drug in PBS, 66.62% and 47.68% of INH and rifampicin, respectively, were diffused within 6 h. The slower release of rifampicin from the lipid layer may be due to slow partitioning and diffusion of rifampicin from the lipid layer to the surrounding aqueous layer. However, when the drug is incorporated in the aqueous layer, the drug may diffuse out due to the concentration gradient as well as due to erosion and biodegradation of the lipid layer[9].

Stability data clearly indicates that liposome encapsulation gives protective effect at least at refrigerated

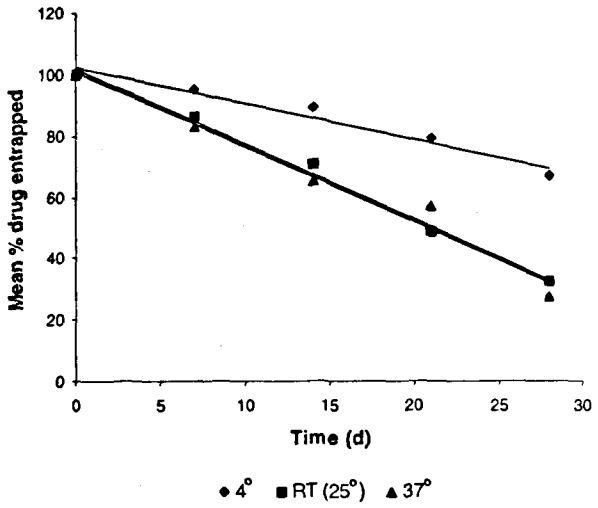

Fig 3: Stability studies of INH from Rif-INH liposomes

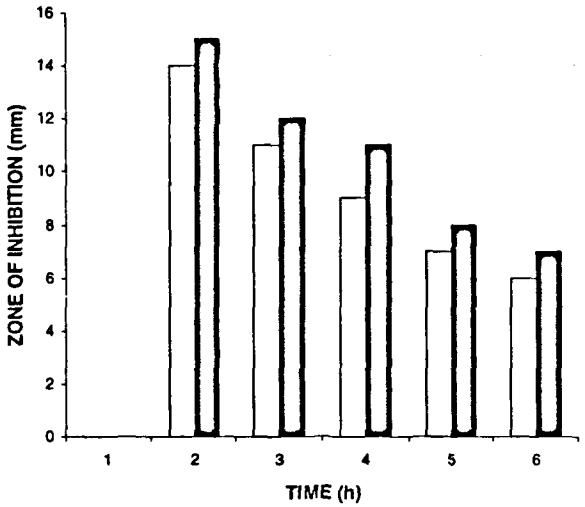

Fig 4: Antimicrobial activity of plain rifampicin and INH (□) and Rif-INH liposomes (■)

condition. However, it is also observed that higher temperature, rate of degradation is higher. This may be due to the presence of lipid materials like cholesterol.

The zone of inhibition was measured for free INH, rifampicin and liposomal INH and rifampicin. The liposomal drugs exhibited a larger zone of inhibition as compared to the encapsulated drugs. The microorganism, i.e. *Staphylococcus aureus*, a gram positive strain, was the most appropriate organism for the estimation of rifampicin and INH with respect to sensitivity and accuracy. Blood samples were collected up to 10 h, only as predicted from the liposomal composition and size, dose selected was 10 mg/kg body weight, according to the published reports[6]. The route of administration was intraperitoneal.

To conclude, rifampicin and INH, the most effective drugs in the treatment of tuberculosis, were successfully encapsulated into liposomal formulation by the lipid layer hydration method. *In vivo* antibacterial study of liposomal drugs exhibited larger zone of inhibition as compared to the free drugs. Thus, it is evident from this study that liposomes could be promising delivery systems for rifampicin and INH with prolonged drug release profiles and better therapeutic effect. Further *in vivo* toxicological and pharmacokinetic studies will be carried out in animal models.

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