Research Paper

Niosomal System for Delivery of Rifampicin to Lymphatics

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Niosomes (nonionic surfactant-based vesicles) containing rifampicin were prepared using various nonionic surfactants of sorbitan ester class and cholesterol in 50:50 percent mol fraction ratio. The drug-entrapped vesicles were characterized for their shape, size, drug entrapment efficiency and *in vitro* release rate. On the basis of *in vitro* characterization, the niosomes showing maximum entrapment and minimum release rate were selected for in vivo performance evaluation. Cumulative percent doses of rifampicin recovered in thoracic lymph following intravenous and intraperitoneal administrations of free rifampicin solution and niosome-encapsulated rifampicin were compared. The study revealed that effective compartmentalisation of the drug took place in the lymphatic system following intraperitoneal administration of niosome-encapsulated rifampicin. Thus rifampicin encapsulated in niosomes could successfully be used for treatment of tuberculosis along lymphatic system.

For effective chemotherapy, an optimal concentration of chemotherapeutic agent must reach the affected tissue/(s) and remain there for a required period of time. Since a number of chemotherapeutic agents are cytotoxic, the presence of drug in the non-diseased tissue/(s) can lead to serious side effects. Encapsulation of drug in vesicular structures can be predicted to prolong the existence of the drug in the systemic circulation and thus enhance penetration into target tissue, and perhaps reduce toxicity if selective uptake can be achieved[1].

The lymphatic system is the second most susceptible site for tuberculosis and ranks after lungs. Targeting of drugs to the lymphatic system is very difficult due to its peculiar nature and position, but by using various routes of administration such as intramuscular[2,3], subcutaneous and intraperitoneal[4-6], significant enhancement in the lymphatic delivery of chemotherapeutic agents has been reported.

Rifampicin is frequently used in the treatment of tuberculosis, a disease widely prevalent, especially in Third World countries, and requiring high dose treatment over a period of 4-6 mo. The causative organism is known to develop resistance if drug blood levels remain below the minimum effective concentration, leading to clinical failure. Rifampicin also has various side effects, such as immunological disturbances, rheumatoid or lupoid syndromes, allergic rashes, eosinophilia, leucopoenia, jaundice and other hepatotoxic manifestations[7].

Niosomes or nonionic surfactant-based vesicles, formed when a mixture of cholesterol and surfactant is hydrated, can entrap solutes, are osmotically active and stable and are similar in terms of their physical properties to liposomes (lipid-based vesicles). Niosomes may overcome the problems associated with liposomes, one of which relates to the chemical instability of the constituent phospholipids. Due to their predisposition to oxidative degradation, phospholipids must be stored and handled in nitrogen atmosphere. The cost and variable purity of natural phospholipids also militate against adoption of liposomes as drug delivery vesicles[8-13]. Niosomes have been used for improving the stability of entrapped drug[14]; for detection of tumours[15]; and to modify the tissue distribution of entrapped harmine[16], influenza antigen[17], nimesulide[18], methotrexate[6,10], doxorubicicin[11], sodium stibogluconate[13], diclofenac sodium[9] and rifampicin[19].

MATERIALS AND METHODS

Rifampicin was procured from Lupin Laboratories Ltd., Aurangabad. Triton-X-100 was procured from Himedia Laboratories Pvt. Ltd., Mumbai. Span-20, Span-40, Span-

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60, Span-80 and Span-85 were procured from Fluka, Germany. Cholesterol was procured from Sigma, St. Louis, MO., USA. Diethyl ether and methanol procured from E. Merck, Mumbai, were used as received; and other reagents were of analytical grade, procured from Qualigens, the chemical division of Glaxo India Ltd., Mumbai.

Preparation of niosomes:

The niosomes were prepared by the method reported by Azmin *et al.*[10] Surfactants and cholesterol (150 µmol) in 50:50 percent mol fraction ratio were dissolved in 10 ml diethyl ether in a 50 ml round-bottom flask, and ether was removed at an ambient temperature (30°) under reduced pressure in a rotatory flash evaporator (Buchi Model, Yorco, New Delhi). The dried film of surfactant was hydrated with occasional shaking for 15 min at 70° on water bath with 5 ml aqueous phase (phosphate buffer saline, pH 7.4) containing 25 mg rifampicin. This suspension was then sonicated for 3 x 30 s to form unilamellar niosomes.

The resultant aqueous dispersions of rifampicin-bearing niosomes were dialysed exhaustively in Cuprophane dialysis tubing against phosphate buffer saline (pH 7.4) to separate the unentrapped rifampicin from the niosome-entrapped rifampicin.

***In vitro* characterization of niosomes:**

The shape and size of the niosomes was studied by an optical microscope using a pre-calibrated ocular eye piece. The entrapment efficiencies were determined by complete dissolution of vesicles using Triton-X-100. The entrapped rifampicin was estimated by digesting a definite quantity of the niosomal suspension with 10% Triton-X-100 for 5 min and centrifuging the resulting solution to get clear supernatant. The supernatant was suitably diluted using phosphate buffer saline and rifampicin estimated using HPLC method reported by Oldfied *et al.*[20]

The *in vitro* release rate was determined using Nesselers cylinder of 50 ml, one end of which was sealed using a circular disc of cellophane membrane (Spectrapore 0.4 µm). Measured amount of niosomes were placed in the cylinder. The cylinder was placed in 500 ml of phosphate buffer saline, pH 7.4, maintained at 37° and aliquots were withdrawn at intervals of 24 h for 5 d. At each sampling time, the volume of receptor compartment was maintained with an equal volume of phosphate buffer saline, pH 7.4. The drug in withdrawn samples was estimated by the reported HPLC method.

***In vivo* lymphatic uptake study:**

The preparation with the composition of Span-85 and cholesterol in 50:50 percent mol fraction ratio was selected for *in vivo* performance evaluation study. Twenty-four Wistar rats of either sex, each weighing about 250 g, were divided into four groups of six each. All animal experiments performed in the present study were approved by the IAEC. The animals were given an oral dose of soyabean oil (4.0 ml/kg) and 1 h later anaesthetised with an injection of urethane (1.2 g/kg). Animals were then dissected and the thoracic duct was cannulated as described by Bollman *et al.*[21] The left femoral artery and the urinary bladder were also cannulated. First and second group animals were administered 0.5 ml of plain drug solution containing 2 mg rifampicin (calculated at the dose level 8 mg/kg) based on human dose intraperitoneally and intravenously respectively, while each animal of the third and fourth group was administered with 0.5-0.7 ml of niosomal formulation (containing 2 mg rifampicin) intravenously and intraperitoneally respectively. The animals were kept at 37±2° in a supine position. Fluid balance was maintained by constant infusion of saline at the rate of 4 mg/h/kg. Lymph was collected periodically over 6 h duration of the study. At the end of 6 h, the animals were sacrificed and the peritoneal cavity was rinsed thrice to recover unabsorbed formulation. All estimations of rifampicin were done by the HPLC method reported by Oldfied[20].

RESULTS AND DISCUSSION

The niosomes were observed as spherical vesicles with smooth surface. The vesicles were discrete and separate with no aggregation or agglomeration. The size of the vesicles was uniform and independent of surfactant, as vesicles of all the surfactants were sonicated to same size. The average size of the vesicles is reported in Table 1.

The percent (of initially taken drug) of drug estimated to be entrapped was noted to decrease progressively for various sorbitan esters used in the order of Span-85>Span-80>Span-60>Span-40>Span-20 (Table 1). This may be explained on the basis of chemical nature of the surfactants. The corresponding HLB values for these surfactants are 1.8, 4.3, 4.7, 6.7 and 8.6 respectively. The lower the HLB number, the more lipophilic is the compound. Thus Span-85 has the highest lipophilicity;

TABLE 1: CHARACTERISTICS OF RIFAMPICIN-LOADED NIOSOMES

Composition of niosomes (Surfactant used)	Mean size of niosomes (μm)	% rifampicin entrapped	% rifampicin released in 120 h
Span - 20	1.8	20.6	80
Span - 40	1.8	25.3	73
Span - 60	1.7	28.5	68
Span - 80	1.8	31.7	60
Span - 85	1.9	35.4	52

therefore, the maximum drug was entrapped in Span-85.

In vitro release rate studies revealed that the cumulative percent rifampicin released was maximum for Span-20-based niosomes and minimum for Span-85-based niosomes (Table 1). The difference in release rate is assumed to be based on lipophilicity of the surfactant. The Span-20, being least lipophilic, would provide easy access to the release media (aqueous phase) to the drug; whereas Span-85, being relatively lipophilic, impedes the easy permeation to the aqueous phase.

The cumulative amount of rifampicin transferred to the thoracic lymph following intravenous and intraperitoneal administration of free drug and niosome-encapsulated drug is shown in fig. 1. As shown in the figure, the maximum lymph concentration achieved was 46.2% of the administered dose, when the drug was administered in the niosomal formulation via intraperitoneal route; while only 7.3% of the administered dose was recovered from the thoracic lymph when the niosomal formulation was administered by intravenous route, and only 13.1% and 11.5% of the administered dose could be recovered from the thoracic lymph after administration of plain drug solution via intraperitoneal and intravenous route respectively. Thus following intraperitoneal administration, effective compartmentalisation of the drug took place in the lymph system. The system thus holds promise for the use in the treatment of tuberculosis along the lymphatic system. However, the initial dose requirement and subsequent supply of drug from niosomes required to achieve the minimum effective concentration need further study.

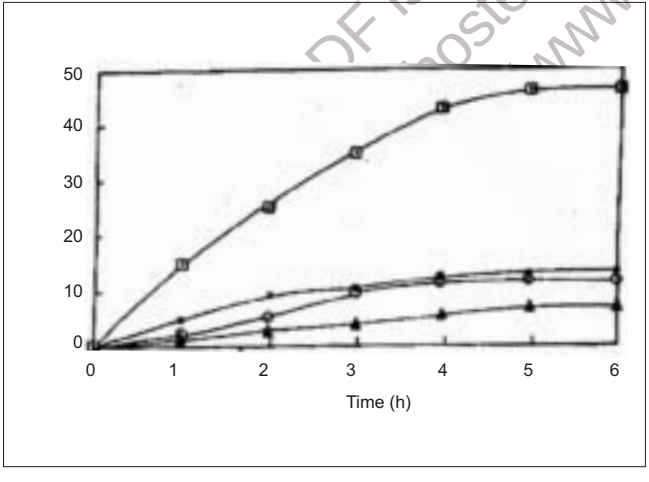

Figure 1: Cumulative percent dose of rifampicin recovered in thoracic lymph
⊘ designates intravenous administration of plain drug solution; • designates intraperitoneal administration of plain drug solution; ▲ designates intravenous administration of niosomal drug and ■ designates intraperitoneal administration of niosomal drug.

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