PREPARATION, CHARACTERIZATION AND TISSUE DISPOSITION OF NIOSOMES CONTAINING ISONIAZID.

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ABSTRACT

Non-ionic surfactant vesicles (or niosomes) are now widely studied as alternates to liposomes. An increasing number of non-ionic surfactant has been found to form vesicles, capable of entrapping hydrophilic and hydrophobic molecules. Isoniazid encapsulated as formulation using ethanol injection method. A different ratio of cholesterol was used. The formulated systems were characterized for in vitro by size distribution analysis, drug entrapment efficiency and drug release profiles. In vivo drug disposition was evaluated in normal, healthy albino rats for niosomal formulation. The size range 2.28 ±0.008(Plain Span 60), 2.311±0.009 (Span60: Cholesterol, 40:50), 2.15±0.002 (span60: Cholesterol 50:50). The entrapment release 74.12% (Plain Span 60), 80.23 %(Span60: Cholesterol, 40:50), 76.26% (span60: Cholesterol, 50:50). In vitro release pattern indicated that about total drug content were released within 48 h. The drug disposition by niosomal drug delivery proved that the drug accumulated in visceral organs (lung, kidney, liver, spleen) was lower than free drug. This proved that niosomal drug delivery system has lesser toxicity than free drug. From the present investigation, it can be concluded that the prepared niosomal drug delivery system of antitubercular agent such as isoniazid has exceptional potential for development into a low dose performed with effective treatment for tuberculosis.

Key Words: *niosome, tissue, tuberculosis*

INTRODUCTION

Non-ionic surfactant vesicles (or niosome) are now widely studied as alternate to liposomes.[1] An increasing number of non-ionic surfactant has been found to form vesicles, capable of entrapping hydrophilic and hydrophobic.[2] Niosomes are uni or multilamellar vesicles formed from synthetic, non-ionic surfactant of alkyl or dialkyl poly glycerol ether class, offering an alternative to liposomes as drug carriers. Niosomes can entrap solutes in a manner analogous to liposomes, are relatively more stable *in vitro* and can improve the stability of entrapped drug as compared with stability in conventional dosage forms.[3] The organism called as *MycobacteriumTuberculosis* causes tuberculosis. Isoniazid is first line antitubercular drug frequently used in the treatment of tuberculosis and requiring a treatment over a period of 4 to 6 months. Isoniazid has various side effects like peripheral neuropath, hepatotoxic.Isoniazid encapsulation by niosomal drug delivery system is to ensure safety to improve the efficacy and to reduce dosing frequency as well as patient compliance. In the present work an attempt was made prepare the niosomes of isoniazid and can continuously deliver therapeutically significant levels of drugs for prolonged tome periods.

EXPERIMJENTAL

Materials and method

Isoniazid (USP) (Lupin laboratories Pvt.Ltd. India), Triton X-100(Sigma St.Louis,MO, USA),Span 60 (Sigma St.Louis,MO, USA),Cholesterol (Sigma St.Louis,MO, USA).diethyl ether

(E.Merk India Ltd) Methanol(E.Merk India Ltd). The materials we used as received and other reagents were of analytical grade.

Preparation of Niosomes: Span 60 and cholesterol was dissolved in 20 ml of diethyl ether, were injected slowly through a 14 gauge needle in to 10ml aqueous phase consisting of 25 mg of isoniazid.

Determination of size distribution: The known amount of drug was dispersed in purified water using spinix shaker and used this suspension for particle analysis was carried out by using Horiba Light scattering particle size analyzer (Horiba Ltd .Japan).

Entrapment efficiency : Entrapment efficiencies were determined by complete disruption of vesicles using Triton X-100. The entrapped drug was estimated by taking a quantity (1.0 ml) of the dialyzed niosomal suspension and digesting for 5 min with 0.1ml tritonX-100. The resulting solution was centrifuged at 1000 rpm for 5min and the supernatant decanted off. The released drug was analyzed using HPLC Method.

HPLC Method: Waters Alliance HPLC system (Milford, USA) with 2695 separations module with auto sampler and column oven was used. Kromasil C18 (250 nm x 4.6 mm i.d 5 µ) column was used as stationary phase and Buffer: Acetonitrile. Buffer (20 mM ammonium acetate, pH adjusted to 6.0 with acetic acid) in the ratio of 99:1 v/v as mobile phase. The mobile phase was filtered through a 0.45 µ membrane filter (sarotorius, Germany) and degassed before analysis. The flow rate was 1ml/min. and the column effluent was monitored at 280nm.

In-vitro* release study*: The isoniazid encapsulated niosomes were separated by gel filtration on sephadex G- 50 powder kept in double distilled water for 48 h for swelling. Now one ml of prepared liposome suspension was placed on the top of the column and elusion was carried out using normal saline. Niosomes encapsulated Isoniazid elutes out first as a slightly dense, white opalescent suspension followed by free drug. Separated niosomes were filled in a dialysis tube to which a sigma dialysis sac was attached to one end. The dialysis tube was suspended in PBS pH (7.4), stirred with a magnetic stirrer and samples were withdrawn at specific time intervals and analyzed using hplc method. To maintain a constant volume, an amount of medium equivalent to the volume of sample withdrawn was added immediately.

***In vivo* distribution studies**: The Albino rats were obtained from animal housing facility, Department of Pharmacology, Kasturba Medical College, MAHE, Manipal (An approved and registered facility under CPCSEA 1998; Registration number 94/CPCSEA/ 1998. The 36 albino male rats weighing 250gms were taken. These rats were divided into two groups containing 18 rats in each group. Group 1 Will received 10 mg/kg body weight[3] of niosomal isoniazid. Prepared µm filters (Millipore, India Ltd) to obtain niosomes< 5µm size. Niosomal suspension used for *in vivo* study was injected intravenously (through tail vein) using appropriate disposal syringe with 22 gauze 1-inch needle. Group II with free drug solution contains 300 mg of isoniazid in PBS. The rats were dissected and the visceral organs (liver, spleen, kidney, lungs) were weighed and macerated. Drug was extracted using PBS from macerated tissues by multiple washings and centrifugations. The supernatants from successive extracts of and organ from each rat were pooled and the drug content was determined. The remaining rats were killed after 6 h and 24 h. The experiment was repeated for free drug solution. The amount of drug in each organ was calculated[4].

RESULTS AND DISCUSSION

Niosomes are non-ionic surfactant based vesicles form and the self assembly of non-ionic amphiphlies in aqueous media results in closed bilayer structure. Niosomes carry all the properties of liposomes except for that they are composed of a surfactant bilayer.

The transmission electron microscopy showed the niosomes prepared by ether injection method were small size, unilamellar, spherical in shape with smooth surface. They were observed as discrete, separate vesicles with no irregularities and aggregation of niosomes.

It was observed that stable vesicles could be prepared without cholesterol. These vesicles displayed the general characteristics of niosomes, although drug was not effectively trapped, thus leading to more rapid leaching of entrapped drug[4].

From the above study it was observed that as the cholesterol content in the vesicles increased, the incorporation of the drug in the vesicles also increased. Cholesterol is the known to increase the rigidity of the niosomal membrane.

In the *in vitro* release studies showed that time taken for 90% leaching of the drug were 21 h for niosomes of plain surfactant and 38 h for niosomes prepared using surfactant and cholesterol ratio 50:50. The cholesterol ratio increased 40:50 the time taken for 90% leaching of drug was 48 h. **Table.1**.

In the i*n vivo* study observed that concentrations of isoniazid after niosomal administration remained in liver which was lower when compared to plain drugs which was administered intravenously, however the concentration of isoniazid in liver was not varied drastically from other visceral organs (kidney, lung, liver and spleen). The liver was the dominant organ in the niosomal disposition because of its larger blood flow and high clearance capacity relative to the other vascular reticulothelial tissues (i.e. spleen). Since the half- life for removal of isoniazid from blood was fast (3 h) a significant fraction of drugs in the liver at 1 h must be located in extracellularly bound niosomes. The isoniazid disposition of niosomes in the liver was lower when compared to the free drug, which was also administered intravenously. It was envisaged that the hepatotoxicity of isoniazid in parenteral administration might be overcome by the niosomal delivery. The initial liver binding was saturated

So the relative liver levels were lower and blood levels were high followed by the increase concentration of isoniazid in kidney was the next highest than that of kidney.Isoniazid was rapidly cleared from the circulation. It may be due to the clearance of the drug from kidney. It was reported that the niosomes that take up the cholesterol-free more than the cholesterol-rich niosomes, whereas the spleen prefers cholesterol rich to cholesterol poor niosomes. Since the niosomes used in this study contain 50 mole% cholesterol, high accumulation in the spleen is expected. It can be concluded that the drug, which reaches to the visceral organs, decreased linearly with time indicating that the continuous elimination of the drug.

CONCLUSION

From the present investigation, it can be concluded that the prepared niosomal drug delivery system of antitubercular agent such as isoniazid has exceptional potential for development into a low dose performed with effective treatment for tuberculosis.

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Table-1: Characterization of niosomal vesicles containing isoniazid

Drug	Niosomal composition	Type of vesicles.	Size µM	Entrapment efficiency	Time Taken for 90%drug release (48 h)
Isoniazid	Plain Span60 (80)	Unilamellar	2.28 ±0.008	74.26%	21
	Span60: Cholesterol: DCP (50:50)	Unilamellar	2.15± 0.002	76.26%	38
	Span60: Cholesterol: DCP (40:50)	Unilamellar	2.311±0 .009	80.23%	48

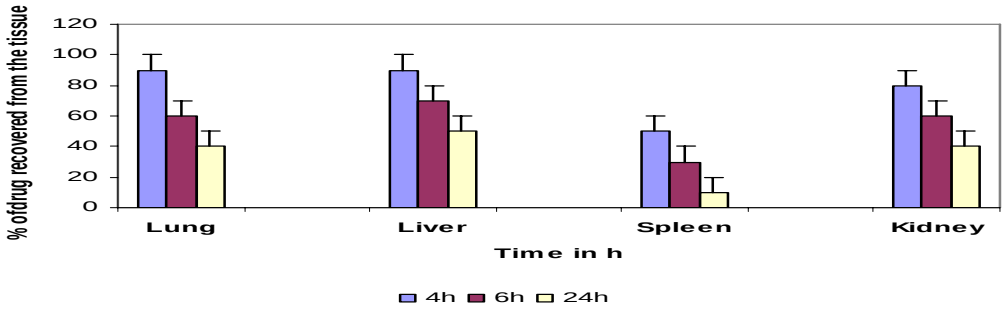

Fig.-1: Tissue disposition of Plain Isoniazid

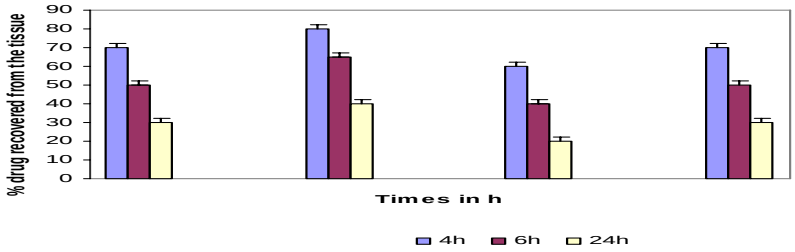

Fig.-2: Tissue disposition of noisome encapsulated Isoniazid

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