original article

JPHS

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In vitro evaluation of itraconazole loaded vesicles prepared from non-ionic surfactants

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ABSTRACT

This study aims to investigate the capability of forming itraconazole containing niosomes with Span 60 and Brij 58 as non-ionic surfactants. Lower cost and higher stability makes niosomes a more suitable choice in comparison with liposomes.The capability to form vesicles as an itraconazole delivery system and the influence of different factors such as type of surfactant and molar ratio of cholesterol/surfactant on the encapsulation efficiency was investigated. The size distribution of vesicles was measured by laser light scattering method. Based on the results, it was observed that the highest encapsulation efficiency and the smallest vesicle size were associated with the formulation composed of Span 60/cholesterol with the molar ratio of 30:70. The observed results were encouraging, and suggested the possibility of using this vesicle system for delivery of itraconazole as a new carrier for treating of fungal infections.

Key words: Niosomes, Non-Ionic Surfactants, Itraconazole, Vesicular Systems.

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1. Introduction

Itraconazole is a hydrophobic drug and belongs to the triazole antifungal agents . Itraconazole is an effective drug against a wide array of funguses. This drug disrupts the cellular membrane of the fungus by inhibiting synthesis of the Ergosterol. Having a fairly low side effect, especially on kidneys, and comprehensive function of this drug makes it a more suitable drug, in comparison with a drug like Amphotericin B. (Katzung, 1998). Niosomes are vesicular structures which are capable of functioning as a delivery system for hydrophilic and hydrophobic drugs (Hao, 2002). Basic structure and formation of the niosomes is exactly similar to that of the liposomes; however, there is a difference between these two. in the structure of the niosomes non-ionic surfactants are used rather than phospholipids, whereasphospholipids are more used in the structure of liposomes. Unlike the liposomes, niosomes are stable against oxidation and hydrolysis and cause less allergic reactions. Non-ionic surfactants are cheap, non-toxic,and can be found in great variety. The amphiphilic structure of the niosomes can lead to increase the permeability, lowering the toxicity, increase absorption, improves solubility, lowers the required dosage and the side-effects of drugs (Palloza, 2006; Gua, 2005). Nowadays, niosomes are used by the pharmaceutical industry in manufacturing skin medications such as anti-psoriasis drugs like Ditranol (Agarwal, 2001), eye medication such as Acetazolamide (Agarwal, 2004) and in cosmetic formulas. Due to high flexibility of the dual layer of niosomes, local application of these vesicular systems increases the penetrability of the medication in the site of infection and makes the drug more effective than its solution form (Agarwal, 2001). This study aims to investigate the capability of forming itraconazole niosomes with Span 60 and Bridge 58 as non-ionic surfactants. For this purpose various formulations of niosomes have been studied with regard to parameters such as morphology, encapsulation efficiency and size distribution.

2. Methodology

2-1. Materials

Itraconazole was kindly supplied by Hetero, India. Span 60 and Brij 58were purchased from Fluka. Cholesterol, chloroform, and isopropyl alcohol in analytical grade were supplied by Merck Co, and Cephadex G25 obtained from GEhealthcare company.

2-2. Preparation of the niosomes

Itraconazole niosomes were prepared using the method of hydration of a lipid layer (Baillie, 1985). In a round-bottom flask, appropriate amounts of non-ionic surfactants along with cholesterol and 10mg of itraconazole were dissolved in 10 ml of chloroform, and then, by using a rotary evaporator at 60oC, the organic solvent was evaporated. Thin layer of film was left to cover the inner walls of the flask. Obtained film hydrated with 10 ml of phosphate buffered saline (PBS, pH 7.4) for one hour at 55 oC . . At the end of this process, the niosomalsuspension was formed.

2-3. Determination of encapsulation efficiency

The drug intended for delivery via the niosomes was separated using column chromatography. The stationary phase used in this process was Cephadex G25 and water was used as the mobile phase (Tabbakhian, 2006; Zhigoltsev, 2005). A predetermined amount of the niosomes suspension was placed on the Cephadex column. After adding water to the column, the drug-containing niosomes migrated from the column and were made slippery using isopropyl alcohol (Baillie, 1986), and then, the amount of the encapsulated drug within the niosomes was determined using UV spectrophotometer at a wave-length of 263 nm.

2-4. Determination of the vesicle size and morphology

The size distribution of the vesicles were determined using laser light diffraction method by Malvern master sizer apparatus(Malvern Master Sizer X, Malvern, UK).

Also obtained niosomal formulations were morphologically investigated using camera attached light microscope (JEM-200 CX, JEOL, Tokyo, Japan)

3. Results

The microscopic observations using a light microscope showed that both types of surfactants used in this study possessed the capability of forming niosomes (Fig. 1).

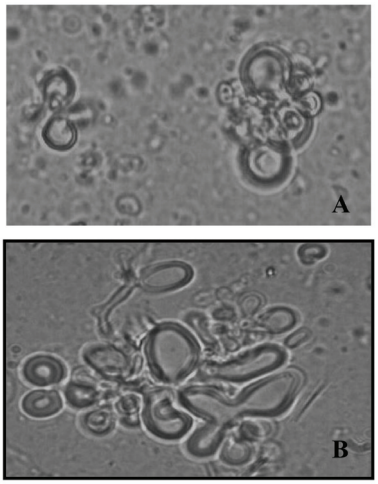

Figure 1. *The light microscopic pictures of niosomal formulations (magnification 10×100).*
A) Niosomes prepared from Span 60: cholesterol with a molar ratio of 30:70.
B) Niosomes prepared from Brij 58: cholesterol with a molar ratio of 40:60.

The drug encapsulation efficiency is considered to be one of the very important parameters. In fact, the function of the medicinal vesicles in pharmaceutical field is associated with the amount of the drug encapsulated within them. In this study, the prepared formulations with span 60, cholesterol with a molar ratio of 30:70, had the highest encapsulation efficiency, and formulation containing Brij 58 and cholesterol with a molar ratio of 40:60, displayed the lowest drug encapsulation efficiency (Tab. 1).

Table 1. *The encapsulation efficiencies of different itraconazole niosomal formulations (mean ± SD, n=3).*.

Formulations	Surfactant/Cholesterol molar ratio	Encapsulation efficiency (%)
Span 60/cholesterol	60:40	34.2 ± 4.5
Span 60/cholesterol	70:30	54.6 ± 1.4
Brij 58/cholesterol	60:40	23.2 ± 1.2
Brij 58/cholesterol	70:30	26.7 ± 0.7

As it is demonstrated in Table 1, when the percentage of cholesterol increased, the encapsulation efficiency of the niosomes decreased. Formulations with the molar ratio of 70:30 displayed higher encapsulation efficiency than the ones with 60:40 molar ratios. The average size of the vesicles, in different formulations, is shown in table 2. Size-wise, as it can be seen, the formulations with Span 60 displayed smaller sizes. Moreover, the formulations with a cholesterol percentage molar ratio of 30 displayed smaller sizes than the ones with a molar ratio of 40.

Table 2. The mean size of different niosomal formulations containing itraconazole (mean ± SD, n=3).

Formulations	Surfactant/Cholesterol molar ratio	Vesicle size (μm)
Span 60/cholesterol	60:40	3.4 ± 0.1
Span 60/cholesterol	70:30	2.3 ± 0.1
Brij 58/cholesterol	60:40	6.6 ± 0.1
Brij 58/cholesterol	70:30	3.9 ± 0.1

4. Discussion

The formulations prepared with Span 60 resulted in the highest encapsulation efficiency, because the structure of Span 60 has a long chain of saturated alkyl. This long saturated alkyl chain allows the molecules of this surfactant to closely and tightly be positioned next to one another and form the niosomic membrane, which leads to a higher level of drug encapsulation efficiency (Hao, 2002; Uchegbu, 1998). The formulations prepared from cholesterol molar ratio of 30 displayed higher encapsulation efficiency than the ones with a cholesterol molar ratio of 40. It can be argued that when the amount of the cholesterol in the lipid layer goes up, a competition between the hydrophobic drug and the cholesterol for being a part of the lipid layer structure is created (Palozza, 2006). The formulations prepared with Span 60 also had a smaller vesicle sizes. Ruckmani suggested that this behavior is due to HLB value of surfactants and in formulation with Span 60 (HLB 4.7), possesses a higher hydrophobic properties than the formulations prepared with Brij 58 (HLB 15.7). As the result of this, Span60 produces vesicles with a smaller size (Ruckmani, 2000). In fact, when the HLB of the surfactant increases, the size of the produced vesicles increases. In this study, it was shown that itraconazole can be encapsulated within the vesiclular systems. The encapsulation efficiency of 54% was achieved using Span 60 and cholesterol at a molar ratio of 70:30. These results exhibited the potential of the niosomal systems to encapsulate itraconazole as an interesting drug carrier for treatment of fungal infections.

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