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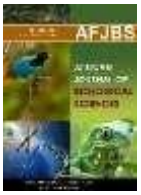

Invivo Pharmacokinetic studies of Itriconazole Drug Loaded Solid Lipid Nanoparticles

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Abstract

In current research, Itraconazole-loaded solid lipid nanoparticles (SLNs) were prepared using glyceryl monostearate (GMS) as a lipid by hot homogenisation method. Itraconazole was selected as a model drug for BCS (Biopharmaceutics Classification system) Class II drugs. Solubility is a major concern for the improvement of oral bioavailability for poorly water soluble drugs as in case of BCS class II. The SLN batches were optimized based on average particle size, drug entrapment efficiency, drug loading and percentage yield. The optimized SLN composition was characterized in terms of particle shape and size, drug entrapment efficiency and drug loading. *In-vivo* antifungal activity against Microsporum canis for optimized batch was carried out using albino rat model for the treatment of dermatophytosis. The bioavailability of drug after oral administration in albino rats was found to be significantly higher as compared to the conventional dosage form of drug. A complete mycological and clinical cure was observed during the study in M. canis infected albino rats. Itraconazole loaded SLNs were administered orally once daily in infected rats for 7 days. It can be concluded from our study that SLNs improves the oral delivery of poorly water soluble drugs like Itraconazole that belongs to BCS Class II drugs.

Keywords: Itraconazole, solid lipid nanoparticles, BCS Class II, antifungal *In-vivo* study

Introduction

The oral route is the most common and convenient method of drug administration to the patients, however, oral administration of drugs often lead to degradation due to the highly acidic gastric environment, enzymes of the mucosa or liver, before they enter the systemic circulation. Beside many highly polar drugs, macro molecular drugs may not be absorbed because of their insufficient poor solubility, lipophilicity, and large molecular weight [1-4]. In drug discovery, the number of drug candidates having low solubility has increased, and approximately 70% of new drug candidates have shown poor aqueous solubility in recent years [5]. Currently, approximately 40% of the marketed immediate release (IR) oral drugs are categorized as practically insoluble [6]. There are many problems arising from the poor solubility of drug candidates in drug research and development. The aqueous solubility of a drug is a critical determinant of its dissolution rate. The limited dissolution rate arising from low solubility frequently results in the low bioavailability of orally administered drugs [7].

The Biopharmaceutics Classification System categorizes drug substances into one of four categories based on their solubility and intestinal permeability, and these four categories are defined as follows: high solubility/high permeability (class I), low solubility/high permeability (class II), high solubility/low permeability (class III), and low solubility/low permeability (class IV). Generally, the bioavailability of a BCS class II drug is rate-limited by its dissolution, so that even a small increase in dissolution rate sometimes results in a large increase in bioavailability [8]. Various approaches to overcome the poor aqueous solubility of drug candidates have been investigated in drug research and development. A promising strategy to overcome these problems involves the development of suitable drug carrier systems.

The antifungal agent Itraconazole is also poorly water soluble drug and belongs to BCS Class II drugs. Its absorption from oral route is also poor [9, 10], as a result, failure in providing effective plasma drug profile on conventional oral administration. The deep-routed micelle of fungal infection necessitate antifungal therapy for prolonged period [11]. The large dose and frequent administration of Itraconazole may lead to contraindicative manifestation [12].

The carrier system serves to protect the drug from premature degradation and inactivation and protects the host from unwanted immunological or pharmacological effects. A wide variety of carriers can be found in nature which serves to bring specific molecules to specific cells. The conventional micellar systems are known to enhance the solubility of poorly absorbed drugs resulting into improved bioavailability [13]. SLNs are colloidal carriers made up of lipids that remain solid at room temperature and body temperature and also offer unique properties such as small size (50-500 nm), large surface area, high drug loading and the interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, nutraceuticals and other materials [14].

Materials and Methods Materials

Itraconazole was obtained as a gift sample from Glaxo SmithKline Pharmaceuticals Ltd., Mumbai. Glyceryl monostearate (GMS) and Tween 80 was purchased from S.D. Fine Chemicals, Mumbai, India. All other chemicals were of reagent grade and purchased from Merck and S.D. Fine Chemicals, Mumbai, India**.**

Preparation of solid lipid nanoparticles Hot homogenisation method

The SLNs were prepared successfully by this method using Glyceryl monostearate (GMS) as lipid carriers, tween 80 as a surfactant and PVA (Polyvinyl alcohol) as a stabilizer. In this method, Glyceryl monostearate was melted above its melting point i.e. 81°C and the drug Itraconazole was dissolved in it. The solution of Tween 80 and PVA was prepared in purified water and heated to the same temperature as that of drug solution. The drug lipid mixture was added drop wise to the hot aqueous solution of surfactant with continuous stirring to make o/w pre-emulsion. The mixture was homogenised at a speed of 2000 rpm for 1 hour. The mixture was then cooled down to the room temperature to give Solid lipid nanoparticles. The resultant mixture was filtered through membrane filter and SLNs were collected.

Result and Discussion

The Itraconazole loaded solid lipid nanoparticles were prepared by hot homogenisation method using 32 factorial design. Total 27 batches were formulated by taking different concentrations of lipid (GMS), surfactant (Tween 80), and stabilizer (Polyvinyl Alcohol). All batches were evaluated for average particle size, entrapment efficiency, drug loading and percentage yield. Based on evaluation, batch GMS-9 was selected as optimized batch on the basis of the smallest particle size, high entrapment efficiency and drug loading.

***In-Vivo* Studies Animals**

Male albino rats (200-250 g) were utilized for *in-vivo* experimental studies. SLNs formulation GMS-9 was selected for *in-vivo* studies on the basis of their average particle size, entrapment efficiency, drug loading and percentage yield. All the animal studies were conducted in accordance with the protocol approved by the Institutional Animal Ethical Committee .

Acute toxicity study

The acute toxicity of Itraconazole was evaluated at doses of 50, 100, 200, 400, 800 and 1600 mg/kg, as per the OECD 423 guideline, and dose of 1600 mg/kg represented toxic indications. Therefore, in agreement with OECD guideline 423, it is expressed as a LD50 cut off value. Doses, 100 mg/kg, bodyweight were preferred for pharmacological inspection by fixed-dose methods [15].

Experimental design

The drug was administered orally as a plain drug suspension and optimized SLNs (GMS-9) in albino rats. The animals were divided into three groups, each group containing five animals. The first group was treated as control and was fed with Phosphate Buffered saline solution (pH 7.4) by oral route. Second and third groups were treated with a single dose of plain Itraconazole suspension in buffered saline solution (pH 7.4) and Itraconazole loaded SLNs (GMS-9) (equivalent to 100 mg Itraconazole per kilogram of body weight) respectively by oral route. Blood samples were withdrawn at 1, 2, 4, 6, 8, 12, and 24 h after dosing. The blood samples were centrifuged, and 100 µl of plasma was separated and immediately frozen until required for analysis. The plasma samples were deproteinized with 100 µl of acetonitrile containing paraphenyl phenol, shaken on a vortex mixture, and centrifuged, and 20 µl of the supernatant was analysed by high-performance liquid chromatography method [16].

Separation was carried out on reversed-phase column, and the column effluent was monitored using ultraviolet detector at 290 nm. The mobile phase was 45% acetonitrile in 0.1 M acetic acid (pH 3.5) at a flow rate of 1 ml/min.

Result and Discussion

In vivo studies revealed that SLNs formulation exhibited a much faster absorption and reached a peak concentration in plasma (Cmax) faster as compared to plain Itraconazole, which exhibited slow absorption. When plain drug was administrated orally, it did not attained higher concentration in the blood and showed higher Tmax due to poor solubility and absorption profile. When the solid lipid nanoparticles were subjected to agar plate diffusion, it was observed that the drug encapsulated in SLNs was effective against M. Canis dermatophytes strains and shows significant zones of inhibition (Table 3). Hence, the pharmacological screening of Itraconazole loaded SLNs found to be improved bioavailability of drug after oral administration in albino rats as compared to the conventional dosage form of drug.

Table 1: Drug plasma concentration time profile studies after oral administration of Plain Itraconazole suspension and Itraconazole loaded SLNs

S. No.	Time (Hrs)	Itraconazole suspension	Itraconazole loaded SLNs
1	1	0.5	1.2
2	2	0.8	3.1
3	4	1.5	2.6
4	6	1.2	2.2
5	8	1.0	2.0
6	12	0.7	1.6
7	24	0.6	1.1

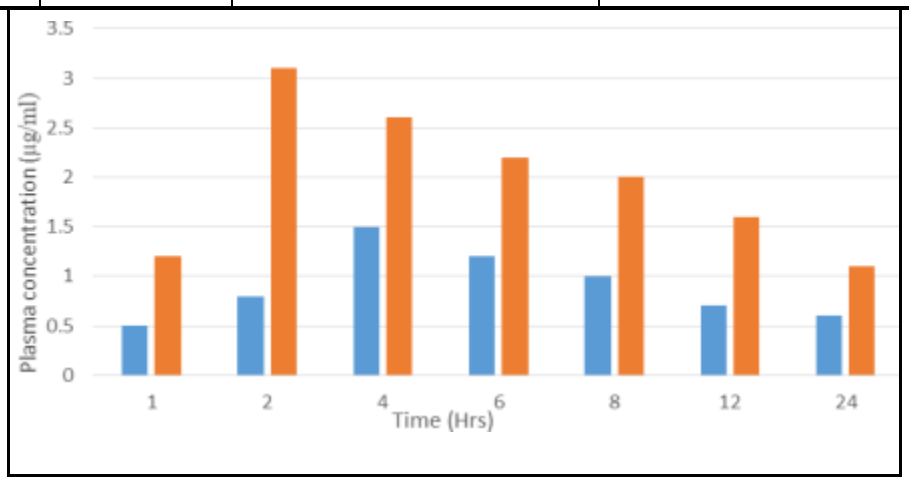

Fig 1: Drug plasma concentration time profile studies after oral administration of Plain Itraconazole suspension and Itraconazole loaded SLNs

Table 2: Pharmacokinetic Data of Plain and Itraconazole loaded SLNs

S. No.	Parameters	Itraconazole suspension	Itraconazole loaded SLNs
1.	C1h µg/ml	0.37	1.00
2.	AUC 0-24 µg/ml h	22.36	41.56
3.	Cmax µg/ml	1.54	2.98
4.	Tmax h	4	2

Table 3: Antifungal efficacy of Itraconazole loaded SLNs against M. Canis fungal strains

Dermatophytes	Colony count (cfu/ml)	Linearity equation	R^2	Zone of Inhibition for SLNs
M. Canis	5.4×10^5	0.234x +1.50	0.992	2.83±0.05

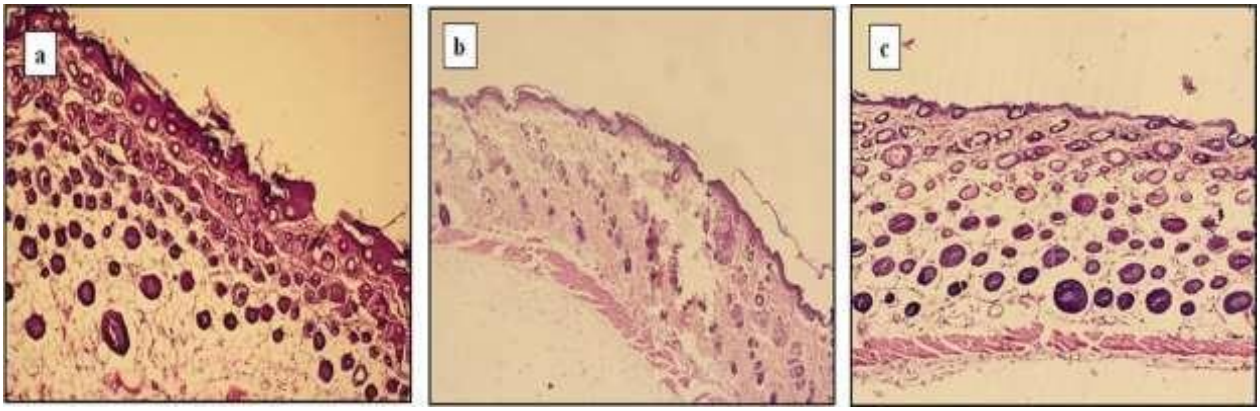

Fig 2: Histological photographs of Rat skin after treatment with (a) phosphate buffered saline pH 7.4 (b) Itraconazole suspension

(c) optimized SLN of Itraconazole

Conclusion

The *In-vivo* pharmacokinetic studies indicated potential of developed Itraconazole loaded solid lipid nanoparticles formulation for faster absorption and augmenting the bioavailability of Itraconazole. The developed system has also shown potential of maintaining higher level of Itraconazole for a longer period of time as compared to plain Itraconazole, which suggested that this system can also act as a depot formulation inside the body. From the present study, it can be concluded that SLNs can be used efficiently for enhancing bioavailability of Itraconazole via oral route.

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