Development and Oral Bioavailability of Self-Emulsifying Formulation of Ketoconazole

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ABSTRACT

Ketoconazole is an imidazole antifungal drug belonging to the class II of Biopharmaceutic Classification System. Maintenance of gastric acidity is essential for adequate dissolution and absorption of ketoconazole. Concurrent administration of antacid and antiulcer preparations decreases the oral absorption of ketoconazole often causing therapeutic failure. The aim of this study was to evaluate whether a self-emulsifying formulation of ketoconazole would be able to overcome the pH dependent dissolution and oral bioavailability. Self-emulsifying drug delivery system (SEDDS) was prepared after selecting the oil, surfactant and co-surfactant by solubility analysis. Optimum ratio of the components was finalized on the basis of drug content, self-emulsification and mean droplet diameter. The effect of pH on dissolution was studied in comparison to the pure drug. Oral bioavailability was determined in comparison to aqueous suspension in rats and the effect of co-administration of ranitidine hydrochloride solution and a commercially available liquid antacid preparation was studied. The optimized formulation containing 20% Capryol 90 and 40% each of Carbitol and Tween 80, exhibited 100% drug release regardless of the pH whereas the pure drug exhibited a highly pH dependent dissolution. The AUC₀⁻²⁴ resulted with oral administration of the SEDDS formulation was about 34%, 43% and 60% higher compared to the aqueous suspension when administered alone, administered with ranitidine and administered with antacid respectively. The results of the present study demonstrate that self-emulsifying formulations can be utilized for oral delivery of weakly basic drugs like ketoconazole which exhibit pH dependent dissolution.

KEYWORDS: SEDDS; Ketoconazole; Dissolution; Oral bioavailability; Gastric pH; Antacids.

Introduction

Formulation of highly lipophilic drugs often poses a challenge in pharmaceutical research as a result of their poor dissolution in aqueous medium. Various techniques have been utilized to increase drug solubility and dissolution of poorly water soluble drugs exhibiting dissolution rate limited absorption. Among those, self-emulsifying formulations are increasingly becoming popular. Self-emulsifying formulations are commonly solutions of drug in a mixture of oil, surfactant and a cosurfactant. These formulations when diluted with aqueous medium with gentle agitation, disperses spontaneously to form fine oil in water emulsion. The rate and extent of absorption of poorly water soluble drug incorporated in self-emulsifying formulations thus increases due to the presence of the drug in soluble form in the gastrointestinal tract offering a large surface area for absorption (Gursoy and Benita, 2004). Apart from that, increase in membrane permeability and possible lymphatic transport due to presence of surfactant and lipidic excipients may also play a role in increase in oral bioavailability of drugs with poor water solubility.

Ketoconazole is an imidazole antifungal drug used orally in chronic mucocutaneous or vaginal candidiasis and fungal infections of skin and fingernails not responding to topical treatment. It is also used in various systemic fungal infections including blastomycosis and candidiasis (Sweetman, 2009). Although it has a wide spectrum of antifungal activity it is not recommended in life threatening fungal infections because of its erratic oral absorption and slow therapeutic response. Absorption of ketoconazole from the gastrointestinal tract is dependent on gastric pH as dissolution of ketoconazole is adequate only at a low pH. Concurrent use of drugs that reduce gastric acidity such as antacids, H₂-antagonists and proton pump inhibitors reduce absorption of ketoconazole (Van Der Meer et al., 1980; Piscitelli et al., 1991). Patients suffering from hypochlorhydria associated with acquired immunodeficiency syndrome (AIDS) also exhibit reduced oral bioavailability of ketoconazole (Lake-Bakaar et al., 1988). Several approaches like solid dispersions (Heo et al., 2005; Kanaujia et al., 2011), in-situ micronization (Rasenack and Muller, 2002) and controlled precipitation
Elder et al., 2007) have been reported to enhance the dissolution and oral bioavailability of ketoconazole.

The aim of this study was to formulate a self-emulsifying drug delivery system (SEDDS) of ketoconazole to overcome the pH dependent dissolution and bioavailability associated with oral administration of the drug. Self-emulsifying formulation containing ketoconazole was prepared and optimized on the basis of physicochemical parameters. Bioavailability of the optimized formulation was studied in rats in comparison to the plain drug. In addition, effect of concurrent administration of antacid and a histamine H2 antagonist on the bioavailability was investigated.

Materials and Methods

Drugs and Chemicals

Ketoconazole was obtained as a gift sample from Johnson & Johnson Ltd. (Mumbai, India). Ranitidine hydrochloride was obtained from Orchev Pharma Pvt Ltd Rajkot, India. Digene liquid (Abbott India Ltd) was purchased over the counter. Labrafac CC (caprylic/capric triglyceride) and Peccol (glyceryl monooleate) was obtained as a gift sample from Colorcon Asia (Goa, India). Labrasol and Capryol 90(propylene glycol monocaprylate) was gifted by Gattefosse (Mumbai, India). Capmul MCM (medium chain mono and di glycerides) was procured as gift sample from Abitech Corporations (Columbus, USA). Propylene glycol, Tween 80, polyethylene glycol 400 (PEG 400), oleic acid and diethylamine were purchased from Qualikems Fine chemicals Pvt. Ltd., New Delhi, India. Cremophor EL was purchased from Sigma Aldrich Inc., Germany. Olive oil, corn oil, cottonseed oil, Carbitol (diethylene glycol monoethyl ether) and castor oil were purchased from Across Organics, Belgium. All chemicals were used as received. Deionized water was prepared by a DQ 3 water purification system from Millipore (Molsheim, France). Methanol and acetonitrile used in the present study was of high performance liquid chromatography (HPLC) grade. All other chemicals were of analytical reagent grade.

Preparation and Evaluation of Ketoconazole Formulation

Solubility studies

The solubility of ketoconazole was determined by adding excess amount of ketoconazole to different oils, surfactants, and co-surfactants in screw capped glass vials. After vortex mixing the mixtures were kept at ambient temperatures for 7 days for equilibration. The equilibrated samples were centrifuged at 5000 rpm for 10 minutes to remove the undissolved ketoconazole. The amount of ketoconazole in the supernatants was quantified by HPLC after diluting suitably with mobile phase.

HPLC analysis of ketoconazole

The concentration of ketoconazole in the samples was determined by a previously reported HPLC method (Huang et al., 1986). The HPLC analysis system consisted of an Adept CE4100 Dual Piston Pump and Adept CE 4201 UV-Visible Variable Wavelength Detector (Cecil, UK). The chromatographic column was a PrincetonSPHER ODS-5 (5 μm) 4.6 mm × 250 mm. A mixture of methanol, acetonirole and diethylamine (60:40:0.05 v/v) was used as the mobile phase at a flow rate of 0.8 ml/min. Loop size was 20 μL and detection was performed at 254 nm. Ketoconazole was eluted at 4 minutes 37±10 seconds with a runtime of 10 minutes.

Preparation of SEDDS formulations

A series of SEDDS formulations were prepared using Capmul MCM and Capryol 90 as oil phase, Tween 80 as surfactant and Carbitol as co-surfactant. Oil content was varied from 90% to 10% w/w and different surfactant to co-surfactant (S/Co-S) ratio (1:1, 2:1, 3:1,1:2 and 2:3) was utilized to prepare the formulations with each oil concentration. Composition representative formulations are shown in Table 1. Ketoconazole sample was taken in screw capped glass vials and dispersed in the oil by vortex mixing. The co-surfactant and the surfactant were then added and again vortex mixed. The mixture was then kept at 60-70°C for 20 minutes. Resultant mixture was centrifuged at 5000 rpm for 10 minutes. The supernatants were collected, filtered through 0.45 μm membrane filter, filled in screw capped glass vials and stored in ambient condition until further use.

### Table 1

Composition of selected SEDDS formulations.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Components (mg)</th>
<th>Ketoconazole content(mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capmul MCM/Capryol 90</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>Tween 80</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Carbitol</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>55.45±3.49</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>90.50±4.50</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>65.56±4.34</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>62.48±3.79</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>75.28±5.15</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>55.39±4.23</td>
</tr>
<tr>
<td>7</td>
<td>300</td>
<td>58.31±2.91</td>
</tr>
<tr>
<td>8</td>
<td>400</td>
<td>69.71±3.98</td>
</tr>
</tbody>
</table>

* Mean±S.D.(n=3)
**Drug content of SEDDS formulations**

Drug content of the prepared formulations were quantified by HPLC after suitable dilution with mobile phase.

**Effect of acidification on drug content**

Effect of acidification on the drug content was studied in selected formulations. Small increments of hydrochloric acid were added with a micropipette to the formulation mixture with constant stirring until a ketoconazole content of 200 mg/ml was achieved.

**Self-emulsification and precipitation assessment**

Evaluation of the self-emulsifying properties of the prepared formulations was performed by visual assessment as previously reported (Kommuru et al., 2001). Visual assessment was performed by addition of 0.1 ml of SEDDS into 250 ml of distilled water taken in a glass beaker at 37°C which was gently stirred magnetically at about 50 rpm. The formulations were then categorized as ‘good’ when the droplets spread easily on the water without any further coalescence within one minute of addition and produced a transparent emulsion. All other cases were categorized as bad. Phase diagrams were constructed identifying the ‘good’ self-emulsifying regions as reported earlier (Kommuru et al., 2001).

Precipitation was evaluated by visual inspection of the resultant emulsion after 24 hours. The formulations were categorized as stable when there was no precipitation at the end of 24 hours.

**Emulsion droplet size analysis**

0.1ml of the self-emulsifying formulation was diluted to 100 ml with water in a volumetric flask and gently mixed by inversion. The resultant emulsion was then subjected to droplet size analysis using Malvern Zetasizer Nano ZS (Worcheshire, UK) with a particle size measurement range of 0.1 nm to 10 µm. All studies were repeated in triplicate.

**In vitro dissolution studies**

The in vitro dissolution test was performed in 900 mL each of 0.1 N hydrochloric acid, acetate buffer pH 4.5 and phosphate buffer pH 6.8 using US Pharmacopoeia XXIII dissolution apparatus 2 at 37°C and 50 rpm. 1 g of the optimized formulation (equivalent to 90 mg ketoconazole) was filled in hard gelatin capsules of size 00 and used for dissolution studies. The procedure was repeated with plain ketoconazole. A volume of 2 ml was withdrawn from the dissolution media at specified time and filtered through 0.22 µm membrane filter. The volume was replaced each time with 2 ml of fresh temperature-equilibrated medium. The amount of ketoconazole in the dissolution samples was quantified by UV-spectrophotometry at 269 nm using a double beam UV-visible spectrophotometer (Schimadzu, UV-1700) after appropriate dilution with 0.1 N hydrochloric acid.

**Oral bioavailability studies**

Male Wistar rats (150±30g) were used for this study. The rats were housed in an animal care facility with a 12 hour light-dark cycle and controlled temperature and humidity. The protocol of the study was approved by the IAEC (992/a/06/CPCSEA, date of approval-13th August, 2010, document no.-RKGIT/CPCSEA/IAEC/Aug.13, 2010/03). All experiments were conducted as per the norms of the Committee for the Purpose of Supervision of Experiments on Animals, India.

The rats were fasted overnight with free access to drinking water. The animals were distributed into six experimental groups (n=4). The rats in the first group received a single oral gavage of the self-emulsifying formulation equivalent to 30mg/kg of ketoconazole. The second group received an aqueous suspension of ketoconazole containing 0.5% sodium carboxymethyl-cellulose (Na-CMC) equivalent to 30 mg/Kg of ketoconazole. The other four groups received similar doses of self-emulsifying formulation or aqueous suspension 20 minutes after receiving either a ranitidine hydrochloride solution (corresponding to 20 mg/kg of ranitidine) or 0.2 ml of a commercially available antacid suspension (Digene liquid, Abbott India Ltd.). During the study, the animals had free access to drinking water and electrolyte solution.

Blood samples (0.2 ml) were collected from lateral tail vein into heparinized tubes pre-dose and at 0.5, 1, 2, 4, 6, 12 and 24 hours post dose. The blood samples were immediately centrifuged at 5000 rpm for 10 minutes and the plasma was harvested. Harvested plasma was put into tapered tubes into which 3 ml of di-ethyl ether was added and vortex mixed for 3 minutes. The tubes were then centrifuged at 3000 rpm for 5 minutes. The organic layer was separated and ether was evaporated by heating in a water bath at 40°C. The residue was then reconstituted with 2 ml mobile phase and ketoconazole content was analyzed by HPLC as described earlier.

**Pharmacokinetic data analysis**

The area under the plasma drug concentration-time curve from zero to 24 hours (AUC0-24) was calculated using the trapezoidal rule. The maximum plasma concentration of drug (Cmax) and the time to reach maximum plasma concentration (Tmax) were obtained directly from the plasma concentration data.

**Statistical Analysis**

The plasma concentration data and pharmacokinetic parameters obtained after administration of different formulations under different conditions were compared by one way analysis of variance (ANOVA) followed by Tukey’s post-hoc analysis and student’s t-test where appropriate. A P value of <0.05 was considered significant.

**Results**

**Solubility studies**

The results of solubility studies are depicted in Fig. 1. Based on the solubility results Carbitol was selected as the co-surfactant, Tween 80 was chosen as the surfactant and Capmul MCM and Capryol 90 both were chosen as oil.
Preparation and evaluation of SEDDS formulations

Self-emulsifying formulations using different ratio of oil, surfactant and co-surfactant were prepared by simple mixing followed by heating. The formulations were optimized on the basis of drug content, self-emulsification and precipitation studies.

Drug content

The drug content of the formulations was found to be dependent on the composition. The oil content of the formulations varied from 90% to 10% w/w with different surfactant/co-surfactant (S/C0-S) ratio. The drug content in all the formulations varied between 44 - 90 mg/ml and is shown in table 1 for selected formulations. Drug content was found to be highest when oil content was 20% with S/Co-S ratio of 1:1.

Self-emulsification and precipitation assessment

Results of self-emulsification studies indicated that formulations containing more than 40% oil had poor self-emulsifying properties. Increasing the co-surfactant concentration hastened the self-emulsification whereas increasing the surfactant concentration the time required for efficient self-emulsification increased owing to gel like layer formation. The compositions corresponding to 'good' self-emulsification are identified in the phase diagram (Fig.2).

Results of precipitation studies indicated that decreasing the oil concentration and increasing the co-surfactant concentration led to increased drug precipitation. Based on drug content, self-emulsification and precipitation studies the formulations containing 20% oil and S/CoS ratio 1:1 were selected for further studies.

Emulsion droplet size analysis

Average droplet diameter of the optimized formulation using Capryol 90 was found to be 98.40 nm with a PDI of 0.345 whereas the average droplet diameter was 245.8 nm with a PDI of 0.598 when Capmul MCM was used as oil.

In vitro dissolution studies

The results of comparative dissolution of the optimized formulation and the pure drug in different dissolution media is shown in Fig. 3. The dissolution of the pure drug was found to be dependent on the pH of the dissolution medium whereas the self-emulsifying formulation released 100% drug within 10 minutes irrespective of the pH.

Oral bioavailability studies

The plasma concentration-time profiles obtained after oral administration of the self-emulsifying formulation and aqueous suspension are shown in Fig. 4. The pharmacokinetic parameters are summarized in Table 2. The time to reach peak plasma concentration was one hour in case of both the formulations when no antacid or ranitidine was co-administered. When ranitidine was administered concurrently there was no change in the Tmax of either of the formulation but with co-administration of antacid formulation the Tmax of the aqueous suspension was 2 hours whereas the Tmax of the self-emulsifying formulation was unchanged. The Cmax and AUCCo24 values were significantly higher in case of the self-emulsifying formulation in all the three cases.
Fig. 2. Ternary phase diagrams showing the good self-emulsifying region: a) using Capryol 90 as oil and b) using capmul MCM as oil.

Fig. 3. Dissolution of SEDDS and pure drug in different dissolution media: a) 0.1 N HCl; b) pH 4.5 acetate buffer; and c) pH 6.8 phosphate buffer (n=3 with 0.5-3.0% variation).
Table 2

Pharmacokinetic parameters after oral administration of ketoconazole (30 mg/Kg) as optimized SEDDS formulation or as an aqueous suspension, alone or administered concurrently with ranitidine hydrochloride solution or commercial antacid preparation.

<table>
<thead>
<tr>
<th>Mean Pharmacokinetic parameters</th>
<th>SEDDS of ketoconazole</th>
<th>Aqueous suspension of ketoconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone</td>
<td>With ranitidine</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;(µg/ml)</td>
<td>72.58 ± 20.08*</td>
<td>81.91 ± 12.48*</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;(h)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt;(µg/ml.h)</td>
<td>971.4 ± 55.36*</td>
<td>771.10 ± 46.60*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.D. (n=4) *p<0.001 compared to the suspension group.
Discussion

Oral bioavailability of ketoconazole is highly dependent on gastric pH. It should not be co-administered with a number of drugs like antimuscarinics and drugs used in the treatment of gastric ulcer due to interference in absorption owing to increase in gastric pH. Although self-emulsifying formulations of griseofulvin (Arida et al., 2007) and itraconazole (Hong et al., 2006; Woo et al., 2008) are reported, self-emulsifying formulation of ketoconazole have not been reported till date. The effect of food on the oral bioavailability of ketoconazole (Skiba et al., 2006) formulated as SEDDS have not been reported yet but the formulations prepared in 0.1 N hydrochloric acid but the formulations prepared in pH 4.5 acetate buffer and pH 6.8 phosphate buffer in comparison to the pure drug. From the comparative dissolution profiles it can be clearly seen that the self-emulsifying formulation releases 100% of the drug load within 5-10 minutes in all the dissolution media whereas the dissolution of the pure drug is pH dependent and decreases considerably with increasing pH of the dissolution medium. Similar pH dependent dissolution of ketoconazole has been reported earlier also (Zhou et al., 2005) and has been attributed to its weakly basic nature (pKₐ 2.94, 6.15). In vivo oral bioavailability studies in rats were performed to confirm whether or not the pH independent in-vitro dissolution of the self-emulsifying formulation also results in pH independent oral absorption.

The effect of pH on oral bioavailability was studied in rats. Ranitidine hydrochloride solution and a commercially available antacid suspension were concurrently administered in four groups of rats with either the SEDDS formulation or ketoconazole aqueous suspension. The results were compared with that obtained with the SEDDS or the suspension alone. The AUC₀₋²⁴ and Cₘₐₓ obtained after administration of the SEDDS were significantly higher (p<0.001) than the ketoconazole suspension in all the three occasions. The AUC₀₋²⁴ resulted with oral administration of the SEDDS formulation was about 34%, 43% and 60% higher compared to the aqueous suspension when administered alone, administered with ranitidine and administered with antacid respectively. The time to reach peak concentration was one hour in case of the SEDDS in all the three cases but with the ketoconazole suspension it was delayed to two hours when co-administered with ranitidine and administered with antacid. It can be explained by the fact that dissolution of the suspension formulation is affected by co-administration of ranitidine and antacid whereas the dissolution of the SEDDS formulation is pH independent and therefore not affected by co-administration of drugs raising gastric pH. Moreover, the fact that AUC₀₋²⁴ resulting from administration of the SEDDS is significantly higher (p<0.001) than the AUC₀₋²⁴ resulting from the administration of the pure drug when administered without any concurrently administered drug, shows that SEDDS formulation results in better oral absorption of ketoconazole, which may be a result of rapid dissolution, availability of large surface area of absorption and involvement of lymphatic transport.

The results of the present study demonstrate that self-emulsifying formulations can be successfully utilized to overcome pH dependent dissolution and oral absorption of weakly basic drugs represented by ketoconazole. Although, the full therapeutic dose of ketoconazole could not be incorporated in a single dosage unit due to stability problems, therapeutic dose of ketoconazole in the form of SEDDS filled capsules can be conveniently administered in divided doses. It has to be assessed further whether higher bioavailability of the self-emulsifying formulation may imply that therapeutic concentrations can be achieved at a lower dose thus also reducing the hepatotoxicity associated with ketoconazole.
Conclusions

Ketoconazole is a BCS class II drug exhibiting pH dependent dissolution and oral absorption. Self-emulsifying formulation of ketoconazole was prepared and evaluated both in vitro and in vivo. The optimized formulation exhibited a small mean droplet diameter on dilution with water and pH independent dissolution in vitro. Oral bioavailability in rats was significantly greater than aqueous suspension of ketoconazole and was mostly unaffected by concomitant administration of ranitidine and antacid. Self-emulsifying formulations can thus be utilized as an alternative to conventional dosage forms to overcome pH dependent oral absorption of weakly basic lipophilic drugs such as ketoconazole. The present formulation needs to be further evaluated in human volunteers.

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References


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