A mixed polymeric micellar formulation of itraconazole: Characteristics, toxicity and pharmacokinetics

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Abstract

A mixed polymeric micelle formulation of itraconazole (ITZ-PM) was prepared using monomethoxy poly(ethylene glycol)-b-poly(lactic acid) and poly(lactic acid) as drug carrier materials. The ITZ-PM formulation remarkably increased the itraconazole solubility up to 15 mg/mL in aqueous media and provided stable solutions at a wide range of concentrations and pH's. In toxicity studies of single and 28-day repeated administrations to rats and dogs, ITZ-PM was well tolerated at dose levels corresponding to clinical doses. The pharmacokinetic profiles of ITZ-PM for itraconazole and its major metabolite, hydroxy-itraconazole, were comparable to those of the cyclodextrin formulations (Sporanox® Injection and Oral Solution) in rats and dogs. These results suggest that ITZ-PM can be an advantageous formulation for both intravenous and oral routes.

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Keywords: Polymeric micelle; Itraconazole; Solubilization; Stability; Pharmacokinetics

1. Introduction

Great efforts have been made to resolve solubility problems associated with poorly water-soluble compounds, namely, drug nanocrystals, nanosuspensions, use of solubilizing excipients, pH adjustment, cosolvents, complexation, microemulsions, self-emulsifying drug delivery systems, micelles, liposomes and chemical conjugates [1–4]. As a carrier for poorly water-soluble drugs, polymeric micelles provide several advantages including escape from reticuloendothelial system (RES) uptake, long circulation in systemic fluid, passive targeting into specific tissues and simple sterilization [5]. Pharmaceutical applications of polymeric micelles, however, are limited due to their instability upon dilution below their critical micellar concentration [6].

The objective of this study was to develop a new polymeric micelle composition of a poorly water-soluble drug, itraconazole, having improved solubility and stability in aqueous media. For the polymeric vehicle, the amphiphilic block copolymer, mPEG-PLA, was chosen based on its proven safety profile in US and Korean clinical studies of Genexol®-PM (Samyang Corporation, Seoul, Korea), in which mPEG-PLA is used as a solubilizing agent for paclitaxel [7] as well as extensive preclinical toxicology studies. The formulation also includes poly(lactic acid) (PLA), the most promising biodegradable polymer in the field of medical devices and pharmaceuticals, for which a number of products are now on the market such as sutures, osteosynthetic devices and drug delivery products [8–10].

Itraconazole is a triazole antifungal agent that is active against a broad spectrum of fungal species and effective for the treatment of both systemic fungal infections and superficial mycoses [11,12]. Like other azole antifungals such as ketoconazole and fluconazole, itraconazole is an ergosterol synthesis inhibitor through potent inhibition of cytochrome P450 (CYP450). Conversion of lanosterol to ergosterol in fungal cell membranes is interrupted by inhibiting the fungal
CYP450 dependant enzyme, lanosterol 14α-methylase [13–15]. Itraconazole undergoes extensive hepatic metabolism to give a large number of metabolites [16]. Hydroxy-itraconazole, the major metabolite of itraconazole, closely resembles the parent drug in chemical structure and has antifungal activity comparable to itraconazole with differences in susceptibility of some fungal species [13,17,18].

Itraconazole, a weakly basic compound (pKa = 3.7), can only be ionized at low pH such as in gastric juice and has an aqueous solubility of 1.8 μg/mL at a pH of 1.2. It is also very lipophilic with a n-octanol/water log partition coefficient of 5.66 at a pH of 8.1 [17,19]. Sporanox® Injection (Janssen Pharmaceutica), the only commercialized product of itraconazole for parental use, achieved dissolution of itraconazole by complexation with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). For empiric therapy, Sporanox® Oral Solution which contains additional inactive ingredients such as sweeteners and flavors follows a 14-day intravenous administration of Sporanox® Injection [12,20,21]. Use of Sporanox® Injection in patients with severe renal dysfunction is limited due to the prolongation of the elimination rate of HP-β-CD. It has also been reported that the excipient HP-β-CD produced pancreatic adenocarcinomas in a rat carcinogenicity study, although its clinical relevance is unknown [20].

In this study, a mixed polymeric micellar formulation of itraconazole (ITZ-PM) was prepared using mPEG-PLA and PLA, its physicochemical characteristics and toxicity for pharmaceutical use were investigated, and pharmacokinetic profiles after intravenous and oral administration were evaluated in comparison with the cyclodextrin formulations of itraconazole (ITZ-CD).

2. Materials and methods

2.1. Materials

Itraconazole was purchased from Choongwae Pharm. Corp. (Seoul, Korea). The cyclodextrin formulations of itraconazole (ITZ-CD: Sporanox® Injection and Sporanox® Oral Solution) were purchased from Janssen Korea Ltd. (Seoul, Korea). Monomethoxy poly(ethylene glycol) was purchased from NOF Corp. (Tokyo, Japan). D,L-lactic acid and D,L-lactide were purchased from Acros Organics (Geel, Belgium) and Purac Biochem (Gorinchem, Netherlands), respectively. Purified water was prepared using a Milli-Q Plus system (Millipore Co., MA, USA). All other chemicals were of HPLC or reagent grade and used without further purification.

2.2. Animals

Sprague–Dawley rats (Sam:TacN(SD), 6 weeks) were purchased from Samtako Korea Inc. (Osan, Korea) and Beagle dogs (6–8 months) were purchased from Beijing Marshall Biotechnology Co., Ltd. (Beijing, China). Animals were maintained in a clean room at a temperature of 19–23 °C with 12 h light and dark cycles and a relative humidity of 40–60%.

2.3. Synthesis of amphiphilic diblock copolymer (mPEG-PLA)

Monomethoxy poly(ethylene glycol)-b-poly(lactic acid) (mPEG-PLA) was synthesized by the ring opening polymerization of D,L-lactide in the presence of mPEG with a catalyst, stannous octoate, as described in the patent application [22]. Briefly, 200 g of mPEG having a molecular weight of 2000 Da was added to a 1 L round-bottomed flask equipped with a mechanical stirrer and a distillation set, and dried at an elevated temperature of 100 °C under vacuum (0.2 mm Hg). Next, 200 g of D,L-lactide and 2.0 g of stannous octoate in 40 mL of toluene were added, and the mixture was stirred for 6 h at 120 °C under nitrogen flow. The reaction product was cooled to room temperature and dissolved in methylene chloride. The solution was poured into cold anhydrous ether (–10–0 °C) to precipitate the diblock copolymer, mPEG-PLA. The precipitated polymer was dried at 30 °C under vacuum (0.1 mm Hg). The number average molecular weights of the mPEG block and the PLA block were 2000 Da and 1800 Da, respectively.

2.4. Synthesis of biodegradable polyesters (PLA-COOH; PLA-COOaNa)

Poly(lactic acid) and its sodium salt (PLA-COOH and PLA-COOaNa) having a number average molecular weight of 1100 Da were synthesized by random copolymerization of D, L-lactic acid, as described previously [6].

2.5. Preparation of itraconazole-containing polymeric micellar formulation (ITZ-PM)

Various compositions of itraconazole-containing polymeric micelles were prepared by a solvent evaporation method [23], and the final ITZ-PM formulation was selected from the results of physicochemical and biological tests. ITZ-PM was prepared as follows: Itraconazole (60 g), mPEG-PLA (300 g), PLA-COOH (180 g) and PLA-COOaNa (180 g) were dissolved in 1.5 L of methylene chloride in a 20 L round-bottomed flask at an elevated temperature of 60 °C using a rotary evaporator to give a clear solution. The solvent was then evaporated under vacuum. Next, 5.5 L of an aqueous solution of lactose (120 g) and sodium chloride (30 g) was added, and the flask was rotated at 100 rpm to form the ITZ-PM in aqueous medium. The solution was filtered using a 0.22 μm PVDF membrane filter and the drug concentration was measured with HPLC to determine the fill weight in each vial. Finally, the filtered solution was filled into vials with an itraconazole content of 200 mg per vial and lyophilized using a Labconco Freeze Dry System equipped with a Stopping Tray Dryer (Labconco Corp., MO, USA).

2.6. Characterization of ITZ-PM

The particle size of ITZ-PM in aqueous media was measured by dynamic light scattering (ELS-8000, Otsuka Electronics Co. Ltd., Osaka, Japan). The itraconazole content was determined using an HPLC system (Hewlett Packard series 1100, Agilent,
CA, USA) equipped with a Vydac C18 column (4.6 mm × 250 mm, 5 μm). The mobile phase consisting of acetonitrile, water, and diethyl amine (60:40:0.05, v/v) was delivered at a flow rate of 1.0 mL/min, and UV detection was utilized at 258 nm. Solubility was measured using unsaturated solutions in WFI (water for injection). After stirring with a shaking water bath (180 rpm) at 25 °C for 24 h, the admixture was centrifuged (5000 rpm, 30 min, 25 °C) and the supernatant was filtered with a 0.22 μm PVDF membrane filter. Solubility was determined by measuring the itraconazole concentration of the filtered solution using HPLC, as described above. For stability testing at different concentrations and pH’s, ITZ-PM and ITZ-CD were diluted in WFI or sodium bicarbonate solution and were stored at 25 °C for 24 h. The amount of itraconazole remaining in solution was assayed using HPLC.

2.7. Hemolysis test

ITZ-PM and ITZ-CD were diluted at concentrations of 1.0–10.0 mg/mL in WFI and normal saline, respectively. To 1450 μL of each solution, 50 μL of rat blood was added. Following incubation at 37 °C for 60 min, the samples were centrifuged (5000 rpm, 10 min) and the amount of cell lysis or released hemoglobin was measured spectrophotometrically at a wavelength of 540 nm.

Table 1

<table>
<thead>
<tr>
<th>Polymer composition</th>
<th>Maximum loading (%)</th>
<th>Solubility (mg/mL)</th>
<th>24-hour stability (%)</th>
<th>Hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPEG-PLA</td>
<td>&lt;1</td>
<td>&lt;0.005</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PLA-COONa</td>
<td>&gt;8</td>
<td>&gt;15 (pH 5)</td>
<td>&gt;95</td>
<td>&gt;30</td>
</tr>
<tr>
<td>mPEG-PLA</td>
<td>&gt;8</td>
<td>&gt;15 (pH 5)</td>
<td>&gt;95</td>
<td>&gt;10</td>
</tr>
<tr>
<td>PLA-COOH</td>
<td>&gt;1</td>
<td>&lt;1 (pH 7)</td>
<td>&gt;99</td>
<td>&lt;5</td>
</tr>
<tr>
<td>PLA-COONa</td>
<td>&gt;12 (pH 7)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*aWeight % of itraconazole in the composition with no drug precipitation.

bAmount of itraconazole remaining in aqueous solutions (3.3 mg/mL) after incubation at 25 °C for 24 h.

cAt a drug concentration of 3.3 mg/mL.

dItraconazole only.

2.8. Animal studies

Toxicity studies were performed at Biotoxtech Co., Ltd. (Cheongwon, Korea) in compliance with GLP Regulations for Nonclinical Laboratory Studies (KFDA Notification No. 2000-63) and Test Guidelines for Safety Evaluation of Drugs (KFDA Notification No. 1999-61). Pharmacokinetic studies in rats and dogs were conducted at Samyang R&D Center and Biotoxtech Co., Ltd., respectively.

Table 2

<table>
<thead>
<tr>
<th>Initial loading (mg)</th>
<th>Loading efficiency (%)</th>
<th>Weight-average diameter (d_a) (nm)</th>
<th>Number-average diameter (d_n) (nm)</th>
<th>Solubility (mg/mL)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITZ mPEG-PLA</td>
<td>200 1000 1200</td>
<td>99.3±0.9</td>
<td>27.7</td>
<td>1.17</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.6</td>
<td>±1.6</td>
<td>±0.1</td>
<td></td>
</tr>
</tbody>
</table>

*aPLA-COOH (600 mg) and PLA-COONa (600 mg).

bWeight % of itraconazole incorporated in ITZ-PM with respect to the initial drug used.

cAt a drug concentration of 3.3 mg/mL.
2.8.1. Single dose toxicity study in rats and dogs

Five male (189–205 g) and five female (138–155 g) Sprague–Dawley (SD) rats were given intravenous injection of ITZ-PM in the tail vein at doses of 15, 30, and 45 mg/kg of itraconazole. Two similarly constituted control groups received saline only and polymer vehicle (dose was matched to the dose of polymer excipients contained in the formulations of ITZ-PM high dose group). Two male (5.3–6.9 kg) and two female (4.9–6.7 kg) beagle dogs were given intravenous infusions of ITZ-PM for 60 min via the cephalic vein at elevating dose levels of 6, 12, and 18 mg/kg of itraconazole with 4-days off-dose. Two similarly constituted control groups received saline only and polymer vehicle of escalating doses (dose was matched to the dose of polymer excipients contained in the formulations of the ITZ-PM group). The clinical signs, mortality, changes in body weight and the necropsy findings of dead and surviving animals were examined.

2.8.2. Repeated dose toxicity study

Animals were divided into 5 groups including saline and vehicle controls, and were dosed once daily for 4 weeks at dose levels of 5.0, 7.5, and 10.0 mg/kg/day as a bolus injection into 12 male and 12 female rats (M: 171–189 g, F: 127–149 g) or as a one-hour infusion into 3 male and 3 female dogs (M: 5.6–8.2 kg, F: 5.7–7.4 kg). Toxicity caused by ITZ-PM was evaluated from clinical signs, changes in body weight, food consumption, ophthalmologic examination, electrocardiography (in dog), urinalysis, hematology, blood chemistry, gross pathology findings and histopathology.

For toxicokinetic study in dogs, blood samples were collected from the cephalic vein with heparin-treated syringes on the first and last (28th) day at designated time intervals: just prior to administration, at 15 and 30 min after the start of infusion, and at 5, 15, and 30 min, 1, 2, 3, 6, 12, and 24 h after the end of infusion. Blood samples were centrifuged (12,000 rpm, 3 min, 4 °C) and the plasma samples were stored at −80 °C until drug analysis.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Single dose toxicity studies with ITZ-PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Route</td>
</tr>
<tr>
<td>Rat</td>
<td>i.v., bolus</td>
</tr>
<tr>
<td>Dog</td>
<td>i.v., infusion</td>
</tr>
</tbody>
</table>

^a Lowest lethal dose.
^b From Ref. [28].

2.8.3. Pharmacokinetics

2.8.3.1. Intravenous administration

2.8.3.1.1. Rats. Male SD rats were anesthetized with Ketamine·HCl (60 mg/kg) and Xylazine·HCl (10 mg/kg) and the carotid artery was cannulated with a 50-gauge polyethylene cannula (Intramedic™, Becton Dickinson, Sweden). ITZ-PM and ITZ-CD were administered intravenously through the tail vein at a dose of 5 mg/kg. Blood samples were collected with heparin-treated syringes at designated time points of 5 and 30 min, 1, 2, 3, 6, 9, and 24 h after drug administration.

2.8.3.1.2. Dogs. Male beagle dogs were given ITZ-PM and ITZ-CD by intravenous infusion for 60 min through cephalic vein using an infusion pump at a dose of 7.5 mg/kg. Blood samples were collected from the cephalic vein with heparin-treated syringes at designated time points: at 30 min after starting infusion, and 0, 5, 15, and 30 min, 1, 2, 3, 6, 12, 24, 36, 48 and 72 h after the end of infusion. Blood samples were centrifuged (12,000 rpm, 3 min, 4 °C) and the plasma samples were stored at −80 °C until drug analysis.

<table>
<thead>
<tr>
<th>Table 5A</th>
<th>Pharmacokinetic parameters of itraconazole on day 1 and day 28 after daily i.v. administration of ITZ-PM for 4 weeks to dogs (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg/day)</td>
<td>Day 1</td>
</tr>
<tr>
<td></td>
<td>C_{max} (μg/mL)</td>
</tr>
<tr>
<td>5.0 Male</td>
<td>3.0±0.7</td>
</tr>
<tr>
<td>Female</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>7.5 Male</td>
<td>3.4±1.4</td>
</tr>
<tr>
<td>Female</td>
<td>2.8±0.2</td>
</tr>
<tr>
<td>10.0 Male</td>
<td>4.2±0.9</td>
</tr>
<tr>
<td>Female</td>
<td>4.2±0.7</td>
</tr>
</tbody>
</table>

^b AUC concentration at the end of infusion.

^b AUC ratio of Day 28 and Day 1 (AUC_{24 h, day 28}/AUC_{24 h, day 1}).

2.8.3.2. Oral administration

2.8.3.2.1. Single dose. Animals were fasted from 12 h before to 3 h after administration. ITZ-PM and ITZ-CD were administered once orally to male beagle dogs using oral sonde at a dose of 7.5 mg/kg. Blood samples were collected from the cephalic vein with heparin-treated syringes at designated time points: 1 and 2 h after drug administration.

<table>
<thead>
<tr>
<th>Table 5B</th>
<th>Pharmacokinetic parameters of hydroxy-itraconazole on day 1 and day 28 after daily i.v. administration of ITZ-PM for 4 weeks in dogs (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg/ day)</td>
<td>Day 1</td>
</tr>
<tr>
<td></td>
<td>C_{max} (μg/mL)</td>
</tr>
<tr>
<td>5.0 Male</td>
<td>0.2±0.0</td>
</tr>
<tr>
<td>Female</td>
<td>0.2±0.0</td>
</tr>
<tr>
<td>7.5 Male</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>Female</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>10.0 Male</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Female</td>
<td>0.5±0.1</td>
</tr>
</tbody>
</table>

^b AUC ratio of Day 28 and Day 1 (AUC_{24 h, day 28}/AUC_{24 h, day 1}).
points of 0.5, 1, 2, 3, 4, 6, 12, 24, 36, 48 and 72 h after drug administration.

2.8.3.3. Multiple doses. Animals were fasted from 12 h before to 3 h after administration.

ITZ-PM and ITZ-CD were administered orally to male beagle dogs using oral sonde at a dose of 7.5 mg/kg once daily for 1 week. Blood samples were collected from the cephalic vein with heparin-treated syringes at designated time intervals: 1 h after administration for 6 days and at 0, 0.5, 1, 2, 3, 4, 6, 12, 24, 36, 48 and 72 h after the first and the last drug administration. Blood samples were centrifuged (12,000 rpm, 3 min, 4 °C) and the plasma samples were stored at −80 °C until drug analysis.

2.8.4. LC/MS/MS analysis of itraconazole and hydroxy-itraconazole

The concentrations of itraconazole and its active metabolite, hydroxy-itraconazole, were determined by LC/MS/MS as follows [24,25]: 100 μL of plasma sample, 100 μL of internal standard (loratadine 2 μg/mL in acetonitrile) and 800 μL of acetonitrile were added into each well of a protein precipitation plate. The mixture was vortexed for 3 min and filtered by vacuum. 10 μL of the solution was injected onto the LC/MS/MS system. A Zorbax XDB-C18 column (2.1 mm × 50 mm, 3.5 μm) was used for the chromatographic separation (Hewlett Packard series 1100, Agilent). The mobile phase consisting of acetonitrile and 0.15% acetic acid (60:40, v/v) was delivered at a flow rate of 0.2 mL/min. MS was carried out on the Quattro Ultima Pt, Micromass equipped with an electrospray ionization in the positive ionization mode. Multiple reaction monitoring transitions of m/z 705.5 → 392.3, 721.5 → 408.3 and 383.3 → 337.2 were used to detect itraconazole, hydroxy-itraconazole and the internal standard (loratadine), respectively. The standard curve of itraconazole and hydroxy-itraconazole was linear over the concentration ranges of 5–50,000 ng/mL and 10–20,000 ng/mL, with correlation coefficient (r²-value) of 0.9984 and 0.9989, respectively. In the within-day and between-day repeated experiments, accuracy and precision were 95–105% and within 8.5%, respectively.

2.8.5. Pharmacokinetic analysis

The area under the concentration–time curve (AUC) was calculated by the trapezoidal rule. The maximum plasma concentration of drug (C_max) and the time to reach maximum plasma concentration (T_max) were obtained from drug concentration data. Terminal half life (T_1/2), area under the concentration–time curve to infinity (AUC_{inf}), plasma clearance (CL), and apparent volume of distribution during terminal phase (V_Z) were calculated by the following relationships:

\[ T_{1/2} = \frac{\ln(2)}{k}, \]
\[ AUC_{inf} = AUC_{last} + \frac{C_{last}}{k}, \]
\[ CL = Dose/AUC_{inf}, \]
\[ V_Z = CL/k = (CL \times T_{1/2})/\ln(2). \]

Table 6

Comparison of pharmacokinetic parameters after single i.v. administration to rats at a dose of 5.0 mg/kg (n=7)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Itraconazole</th>
<th>Hydroxy-itraconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_{min} (μg/mL)</td>
<td>AUC_{24 h} (μg h/mL)</td>
</tr>
<tr>
<td>ITZ-PM</td>
<td>1.5±0.2</td>
<td>3.8±0.4</td>
</tr>
<tr>
<td>ITZ-CD</td>
<td>1.3±0.2</td>
<td>3.8±0.9</td>
</tr>
</tbody>
</table>

*aDrug concentration at 5 min post-injection.
2.8.6. Statistical analysis

A student’s t-test was used for comparison of the pharmacokinetic parameters in different formulations and a p value of less than 0.05 was considered statistically significant. All results were expressed as the mean ± one standard deviation (SD).

3. Results and discussion

3.1. Physicochemical characteristics of ITZ-PM

The ITZ-PM formulation was selected from various polymer compositions by evaluation of physicochemical properties and hemolytic potential in rat blood (Table 1). When mPEG-PLA was used, the maximum loading of itraconazole with no precipitation was less than 1%, and the aqueous solubility of this composition was lower than 0.005 mg/mL. PLA-COOH having a molecular weight of 1100 Da formed polymeric micelles in water and showed increased solubility and stability, but the drug precipitated at pH 7 and severe hemolysis was observed. The best result was obtained from a polymer composition comprised of mPEG-PLA, PLA-COOH and PLA-COONa. It is considered that a polymer–drug complex formed by the reaction between the carboxyl group of PLA and the amine or triazole group of itraconazole is entrapped into the hydrophobic core of the micelles due to the high affinity between the PLA in core of the micelles and the PLA–drug complex.

Itraconazole was effectively incorporated in the polymeric micelle with a loading efficiency of 99.3%. In aqueous medium and a drug concentration of 10 mg/mL, the weight-average and number-average diameters of ITZ-PM were 27.7 and 23.5 nm, respectively (Table 2 and Fig. 1). The aqueous solubility of itraconazole (<0.001 mg/mL) was remarkably increased in the mixed polymeric micelle. After reconstitution in water, ITZ-PM provided an itraconazole solubility of 15.4 mg/mL. Stability upon reconstitution and dilution was evaluated at concentrations appropriate for clinical intravenous administration. Stable solutions without any precipitation were maintained at a wide range of concentrations (1–10 mg/mL) for longer than 24 h. On the contrary, as shown in Fig. 2, dilution of ITZ-CD at concentrations below 3.3 mg/mL led to the formation of precipitate. In order to obtain a stable admixture of ITZ-CD, it is critical to maintain a 3.33 mg/mL of itraconazole [20].

The pH of the ITZ-PM solution after reconstitution was approximately 5.2 and the aqueous solution of ITZ-PM was stable at pH’s up to 7. On the other hand, ITZ-CD formulations are supplied as solutions of 10 mg/mL and their solution stability greatly depends on the pH and the concentration at dilution due to the physicochemical properties of the itraconazole/2-HP-β-CD inclusion complex in aqueous propylene glycol solution [26,27]. Taken together, the physicochemical properties of ITZ-PM summarized in Table 2 indicate that the ITZ-PM can be an advantageous pharmaceutical formulation.

3.2. Hemolytic potential

Hemolytic potential was evaluated in rat blood to ensure hemocompatibility of ITZ-PM. As shown in Table 3, when ITZ-PM solutions of 10.0, 3.3 and 1.0 mg/mL were in contact with red blood cells, the percent hemolysis was 16.7%, 2.2% and 1.8%, respectively. On the other hand, ITZ-CD caused serious hemolysis even at the concentration of 3.3 mg/mL (66% hemolysis), which is the required concentration for clinical i.v. infusion. The results of solution stability and hemolytic potential indicate that ITZ-PM can be administered intravenously at a wide range of drug concentrations so that a precise dilution is not required.

3.3. Toxicology

3.3.1. Single dose toxicity study

The acute toxicity of ITZ-PM was investigated following i.v. bolus injection to SD rats and 1-hour infusion to beagle dogs. At a dose of 45 mg/kg in male rats, 2 animals died. No mortality was observed at doses of 30 mg/kg in male rats, 45 mg/kg in male rats, and 30 mg/kg in beagle dogs. The results of solution stability and hemolytic potential indicate that ITZ-PM can be administered intravenously at a wide range of drug concentrations so that a precise dilution is not required.

3.3.2. Continuous administration toxicity study

The continuous administration toxicity of ITZ-PM was evaluated in SD rats and beagle dogs. ITZ-CD caused serious hemolysis even at the concentration of 3.3 mg/mL (66% hemolysis), which is the required concentration for clinical i.v. infusion. The results of solution stability and hemolytic potential indicate that ITZ-PM can be administered intravenously at a wide range of drug concentrations so that a precise dilution is not required.

3.3.3. Oral administration toxicity study

The oral administration toxicity of ITZ-PM was evaluated in SD rats and beagle dogs. ITZ-CD caused serious hemolysis even at the concentration of 3.3 mg/mL (66% hemolysis), which is the required concentration for clinical i.v. infusion. The results of solution stability and hemolytic potential indicate that ITZ-PM can be administered intravenously at a wide range of drug concentrations so that a precise dilution is not required.

3.3.4. Long-term administration toxicity study

The long-term administration toxicity of ITZ-PM was evaluated in SD rats and beagle dogs. ITZ-CD caused serious hemolysis even at the concentration of 3.3 mg/mL (66% hemolysis), which is the required concentration for clinical i.v. infusion. The results of solution stability and hemolytic potential indicate that ITZ-PM can be administered intravenously at a wide range of drug concentrations so that a precise dilution is not required.

Table 7

Comparison of pharmacokinetic parameters after 1-hour i.v. infusion at a dose of 7.5 mg/kg to dogs (n=3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Itraconazole</th>
<th>Hydroxy-itraconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;end&lt;/sub&gt; (μg/mL)</td>
<td>AUC&lt;sub&gt;72 h&lt;/sub&gt; (μg·h/mL)</td>
</tr>
<tr>
<td>ITZ-PM</td>
<td>3.3±1.0</td>
<td>19.4±3.3</td>
</tr>
<tr>
<td>ITZ-CD</td>
<td>4.3±0.4</td>
<td>23.2±1.8</td>
</tr>
</tbody>
</table>

* Drug concentration at the end of infusion.
* Significantly different from ITZ-CD (p<0.05).
female rats and 18 mg/kg in dogs. Clinical signs observed were paleness, hypoactivity and decreased respiration in rats at a dose of 45 mg/kg. Although some clinical signs such as anorexia, diarrhea, and soft feces were observed in both control and treatment groups in dogs, dose-dependency was not observed, and thus, they were not considered to be treatment-related responses. There were no abnormal body weight changes in all the animal groups. In necropsy, no macroscopic abnormalities were observed in both rats and dogs. As summarized in Table 4, the single dose toxicity of ITZ-PM was somewhat reduced compared to ITZ-CD [28] probably due to the superior safety profile of the polymer vehicle of ITZ-PM, mPEG-PLA and PLA.

3.3.2. Repeated dose toxicity study

The repeated dose toxicity of ITZ-PM was studied with once daily doses of 5.0, 7.5 and 10.0 mg/kg/day for 28 days as an i.v. bolus injection to SD rats and a 1-hour infusion to beagle dogs.

In the toxicity studies in rats, no clinical findings including mortality were observed. Treatment with ITZ-PM did not affect body weight changes and food consumption. No toxic effects were noted in ophthalmologic examination, urinalysis and hematology. A slight decrease in hematocrit and hemoglobin concentration was seen within the normal range and the values were not considered to have toxicological relevance. A dose-related increase in total cholesterol was observed, but this is known to be a rat-specific effect [29,30]. Increase in liver weights was seen in both male and female rats, but no relevant effect on liver function was observed in hematology, blood chemistry and histopathological examination. Weights of adrenals increased dose-dependently in female rats and hyperplasia of zona fasciculate was observed in histopathological examination. The changes in adrenal cortex were typical lesions caused by itraconazole [29].

In dogs, no toxic effects were observed at doses of 5.0 mg/kg and 7.5 mg/kg. On the scheduled necropsy day, one female died and one male was moribund in the 10.0 mg/kg groups. Treatment-related toxicity was observed at 10.0 mg/kg, as evidenced by anorexia, decrease in body weight gains, altered blood parameters, fatty liver, hyperplasia of the adrenal cortex and lymphocyte depletion in lymphatic tissues. No difference was seen in toxic response between males and females and the NOAEL (no observed adverse effect level) for repeated daily dose of ITZ-PM in dogs was considered to be 7.5 mg/kg. Similar results were reported from toxicity studies of Sporanox® Injection [28]. In general, the changes observed in the rat and dog studies were expected from the toxicology profiles of itraconazole alone. No toxic signs related to the polymer vehicle of ITZ-PM were noted.

In the toxicokinetic study, plasma concentrations and AUC values of itraconazole and hydroxy-itraconazole increased dose-proportionally over the 5.0- to 10.0-mg/kg/day dose levels on the first day of administration. Further, on day 28, the AUC$_{24}$ and C$_{max}$ values of itraconazole and hydroxy-itraconazole increased dose proportionally over the dose range from 5 to 7.5 mg/kg but they tended to increase non-proportionally at 10 mg/kg. As summarized in Tables 5A and 5B), accumulation ratios of itraconazole and hydroxy-itraconazole increased from 1.6–2.1 and 6.1–7.7 at the 5.0 mg/kg dose to 2.9–3.8 and 11.6–17.1 at the 10.0 mg/kg dose, respectively. No difference was found between the male and female groups.

3.4. Pharmacokinetics

3.4.1. Intravenous administration to rats and dogs

The average plasma concentration vs. time curve of itraconazole and hydroxy-itraconazole and their pharmacokinetic parameters after i.v. bolus injections of ITZ-PM and ITZ-CD to SD rats (5.0 mg/kg) and 1-hour infusions to beagle dogs (7.5 mg/kg) were compared.

After intravenous administration of both ITZ-PM and ITZ-CD to rats, itraconazole showed a rapid distribution phase of less than 1 h and its terminal elimination half-life was 7 to 8 h. Hydroxy-itraconazole was detected from the first blood sampling time, 5 min, and reached its peak at 6–9 h. There were no differences in the pharmacokinetic parameters of

<table>
<thead>
<tr>
<th>Table 8</th>
<th>Comparison of pharmacokinetic parameters after single oral administration to dogs at a dose of 7.5 mg/kg (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Itraconazole</td>
</tr>
<tr>
<td>C$_{max}$ ($\mu$g/mL)</td>
<td>T$_{max}$ (h)</td>
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<tr>
<td>ITZ-PM</td>
<td>0.91±0.08</td>
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<tr>
<td>ITZ-CD</td>
<td>0.73±0.27</td>
</tr>
</tbody>
</table>

*Oral Bioavailability= AUC$_{72}$ h(oral)/AUC$_{72}$ h(iv). AUC$_{72}$ h(iv) values of ITZ-PM and ITZ-CD were 19.4 and 23.2, respectively (Table 6). *Significantly different from ITZ-CD (* p<0.05; ** p<0.01).

<table>
<thead>
<tr>
<th>Table 9</th>
<th>Comparison of pharmacokinetic parameters on day 7 after daily oral administration at a dose of 7.5 mg/kg/day to dogs (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Itraconazole</td>
</tr>
<tr>
<td>C$_{max}$ ($\mu$g/mL)</td>
<td>T$_{max}$ (h)</td>
</tr>
<tr>
<td>ITZ-PM</td>
<td>1.58±0.30</td>
</tr>
<tr>
<td>ITZ-CD</td>
<td>1.46±0.54</td>
</tr>
</tbody>
</table>
itraconazole and hydroxy-itraconazole between the two formulations (Fig. 3 and Table 6).

When ITZ-PM and ITZ-CD were infused to dogs, itraconazole showed a longer distribution phase of up to 6 h with a terminal elimination half-life of 35 to 40 h. As shown in Fig. 4 and Table 7), pharmacokinetic profiles of both itraconazole and hydroxy-itraconazole in ITZ-PM was similar to those in ITZ-CD. Moreover, the AUC ratios of hydroxy-itraconazole to itraconazole in both formulations were similar, 1.3 in rats and 0.7 in dogs, respectively.

3.4.2. Single and multiple oral administration to dogs

Pharmacokinetic profiles following single and 7 daily administrations of ITZ-PM and ITZ-CD to beagle dogs were compared. After single oral administration of ITZ-PM and ITZ-CD to dogs at a dose of 7.5 mg/kg, itraconazole was absorbed rapidly, reached its peak plasma concentration at 1.2–1.3 h, and declined with a mean terminal $T_{1/2}$ of 43–45 h. The oral bioavailability (BA) of ITZ-PM and Sporanox® Oral Solution were 54.1% and 40.1%, respectively, based on their respective $AUC_{72h}$ of itraconazole after an intravenous dose of 7.5 mg/kg. On the other hand, hydroxy-itraconazole was detected from the first blood sampling time, 30 min, and reached its peak at 13–16 h. No difference was shown in the pharmacokinetic parameters of itraconazole between the two formulations whereas $C_{max}$ and $AUC_{72h}$ of hydroxy-itraconazole in ITZ-PM were slightly higher than those in ITZ-CD (Fig. 5 and Table 8). The difference in the pharmacokinetic parameters of hydroxy-itraconazole may not be significant since this difference was not seen following multiple administrations, as shown in Table 9).

After once daily repeated dosing of ITZ-PM and ITZ-CD to dogs, the plasma concentration of itraconazole reached steady-state within 5 days and the trough concentration was maintained above the target concentration of 0.5 μg/mL [12]. On day 7, after multiple intravenous infusions, both formulations exhibited similar pharmacokinetic profiles as shown in Figs. 6 and 7 and Table 9). As compared with the plasma levels of itraconazole and hydroxy-itraconazole after single administration of both formulations, the levels after the last dose of multiple administrations increased to the same extent in both formulations. Finally, the $C_{max}$ and $AUC_{72h}$ increased by 1.9 and 4.0 times the itraconazole and 4.4 and 4.7 times with hydroxy-itraconazole, respectively.

4. Conclusion

A mixed polymeric micelle formulation of itraconazole (ITZ-PM) was prepared using monomethoxy poly(ethylene glycol)-b-poly(lactic acid) and poly(lactic acid) as drug carrier materials. Its physicochemical properties and safety profile showed enhanced solution stability at a wide range of concentrations and pH’s with good tolerability of the polymer vehicle. The pharmacokinetic profiles after intravenous and oral administration of ITZ-PM to rats and dogs were compared with the cyclodextrin formulation of itraconazole (ITZ-CD). The results showed similar pharmacokinetic profiles between the two formulations, indicating that the use of the polymer vehicle, mPEG-PLA and PLA, in the ITZ-PM formulation had no influence on the pharmacokinetics of itraconazole and hydroxy-itraconazole. These results indicate that ITZ-PM appears to be an advantageous formulation for intravenous and oral routes, and the efficacy of ITZ-PM will be further studied.
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References