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Development of a binary lipid nanoparticles formulation of itraconazole for parenteral administration and controlled release

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ABSTRACT

The principal aim of this study was to develop an intravenous formulation of itraconazole (ITZ) using lipid nanoparticles based on binary mixture of liquid and solid lipids. Lipid nanoparticles were developed to provide the controlled release of ITZ as well as to improve the solubility of ITZ. Lipid nanoparticles were prepared with tristearin as a solid lipid, triolein as a liquid lipid, and a surfactant mixture of eggPC, Tween 80 and DSPE-PEG_{2000}. ITZ was incorporated at the concentration of 20 mg/g. Lipid nanoparticles were manufactured by high-pressure homogenization method. The particle size and polydispersity index (PI) of lipid nanoparticles were below 280 nm and 0.2, respectively. Zeta potentials and incorporation efficiencies of lipid nanoparticles were around −30 mV and above 80%, respectively. Lipid nanoparticles containing 1% of liquid lipid showed the smallest particles size and the highest incorporation efficiency. Results from SEM, DSC and PXRD revealed that ITZ in lipid nanoparticles exists in an amorphous state. Release rates were increased as the amount of liquid lipid in lipid core increased, demonstrating that the release of ITZ from lipid nanoparticles could be controlled by modulation of the amount of liquid lipid in lipid core. Pharmacokinetic studies were performed after intravenous administration of lipid nanoparticles in rats at the dose of 5 mg/kg. The plasma concentration of ITZ was prolonged after intravenous administration of lipid nanoparticles. It is concluded that binary lipid nanoparticles could control the release and pharmacokinetic parameters of ITZ.

1. Introduction

Itraconazole (ITZ) is a triazole antifungal agent against a broad spectrum of fungal species and effective for the treatment of both systemic fungal infections and superficial mycoses (Beule and Gestel, 2001; Boogaerts et al., 2001). In case of candidemia and other opportunistic mycoses, the necessity of an intravenous formulation of ITZ has been raised since many neutropenic and other immunocompromised patients have difficulty in swallowing the oral capsule formulation of the drug.

However, the development of intravenous formulation is difficult due to the high lipophilicity with an *n*-octanol/water partition of log 5.66 at pH 8.1 (Fromtling, 1987; Jung et al., 1999) and low aqueous solubility of approximately 1 ng/ml at neutral pH and approximately 4 mg/ml at pH 1 (Peeters et al., 2002). Sporanox® I.V. is the only commercially available intravenous formulation of ITZ solubilized by hydroxylpropyl-β-cyclodetrin (HP-β-CD), which facilitates the establishment of high and dependable levels of the active compound in plasma. It has been successfully used in the treatment of severe necrotizing pneumonias, invasive pulmonary *aspergillosis* and so on (Groll et al., 2002; Picardi et al., 2003). Nevertheless, Sporanox® is not allowed to be used in patients with impaired renal function. It is not because of the toxicity of the drug itself, but the adjuvant HP-β-CD. Each milliliter of Sporanox® contains 10 mg of ITZ solubilized by 400 mg of HP-β-CD as an inclusion complex. Following a single intravenous dose of 200 mg Sporanox® to the subjects with severe renal impairment, clearance of HP-β-CD was 6-fold reduced compared with subjects with normal renal function. Although its clinical relevance is unknown, it has been reported that HP-β-CD produced pancreatic adenocarcinoma in rat carcinogenicity study (Physician's Desk Reference, 2005). Hence, a development of intravenous formulation of ITZ without HP-β-CD is of great potential. In order to solve the problem of HP-β-CD, many parenteral formulations of ITZ such as emulsion (Akkar and Müller, 2003), polymeric micelle (Yi et al., 2007), nanosuspension (Rabinow et al., 2007) and albumin bound nanoparticles (Chen et al., 2008) have been studied without HP-β-CD.

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Table 1
Composition of lipid nanoparticles.

Formulations	ITZ (mg/g)	Triolein (mg/g)	Tristearin (mg/g)	eggPC (mg/g)	Tween80 (mg/g)	DSPE-PEG_{2000} (mg/g)
F0	20	0	100	75	15	10
F10	20	10	90	75	15	10
F20	20	20	80	75	15	10
F50	20	50	50	75	15	10

Lipid nanoparticles are named after the content of triolein as liquid lipid in each formulation.

The classical lipid nanoparticles that have been proposed for drug delivery are composed of solid lipids. A distinct advantage of solid lipid nanoparticles (SLN) over polymeric nanoparticles is the fact that the lipid matrix is made from physiologically tolerated lipid components, which decreases the potential acute and chronic toxicity (Müller et al., 2000). SLN based on pure triglycerides like tripalmitate exhibit limited drug payloads and drug expulsion from the crystal lattice (Westesen et al., 1997). Using complex glycerides like hard fats as a matrix for SLN, incorporation of lipophilic drugs is facilitated (Jenning et al., 2000). However, these hard fat SLN reveal a tendency to form supercooled melts instead of solid particles. Even if solidified at room temperature these particles melt at body temperature. Therefore, these particles are not suited for controlled release applications.

The capabilities of including drug molecules in the lattice of crystalline lipids are often limited whereas liquid oils normally show considerable higher solubility for lipophilic drugs. Mixtures of liquid and solid lipids are not restricted to these semi-synthetic materials but can be found as well in naturally occurring products like milk, cream or cocoa butter (Precht, 1988). Similarly, conventional SLN were supplemented with liquid oils to resolve the issues of insolubility as well as controlled release (Jenning et al., 2000). Nanoparticles with low oil concentrations showed sustained release profiles as well as improved drug encapsulation by lipid particles supplemented with oily constituents.

Here, we developed binary lipid nanoparticles to increase the solubility of ITZ and to control the release from lipid nanoparticles. The physicochemical characterization, in vitro release behavior, and stability of ITZ-loaded binary lipid nanoparticles were evaluated. Moreover, pharmacokinetics of ITZ-loaded binary lipid nanoparticles were investigated in rats.

2. Materials and methods

2.1. Materials

Triolein, tristearin and Tween 80 (polyoxyethylene sorbitan monooleate) were purchased from Sigma (St. Louis, MO, USA). Egg yolk L-a-phosphatidylcholine (eggPC) and 1,2-distearoyl-sn-glycero-3-phosphatidyl-ethanolamine-N-[methoxy(polyethyleneglycol)-2000] (DSPE-PEG_{2000}) were provided by Avanti Polar lipids (Alabaster, AL, USA). The degree of purity of the all lipid excipients used in this study was over 99% as supplied by the vendor. ITZ and miconazole were kindly donated from Hanmi Pharm. (Seoul, Korea). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of lipid nanoparticles

Lipid nanoparticles were prepared by melt homogenization method (Schwarz et al., 1994). To prepare liquid–solid lipid nanoparticles, tristearin, triolein, eggPC, Tween 80, DSPE-PEG_{2000} and ITZ were mixed and put in water bath with the temperature above the melting point of tristearin. Tristearin was used as solid lipid. Trioleins was used as a liquid lipid because it has the same chain length of fatty acid with the solid lipid, tristearin. The composition of liquid–solid lipid nanoparticles is presented in Table 1. After a few minutes of incubation, preheated water for injection was added to the lipid melts (2 g of final total weight). These dispersions were sonicated for 1 h in bath type sonicator (Branson® ultrasonic cleaner, 3210R-DTH, Branson Ultrasonics Corp., CT, USA) until crude and milky emulsions were obtained. Since physical energy should be added in the manufacturing process of emulsion to maintain the state of lipid as liquid, bath type sonicator is selected due to the ease of experimental application in laboratory. The resultant crude emulsions were homogenized using a high-pressure homogenizer (Emulsiflex® EF-B3, Avestin Inc., Canada) wired with heating tape (Thermolyne®, Barnstead International, USA). After homogenization, the hot dispersions were immediately filtered through a 1 μm membrane filter to remove free ITZ. Lipid nanoparticles were produced by subsequent cooling of hot fine emulsions in liquid nitrogen. Lipid nanoparticles were then thawed at room temperature and stored at 4 °C for further studies.

2.3. Measurement of particle size and zeta potential

The mean particle size and polydispersity index (PI) of lipid nanoparticles were determined by dynamic light scattering method using electrophoretic light scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan) with a fixed angle of 90° at room temperature. Prior to measurement, lipid nanoparticles were diluted with filtered deionized water. The system was used in the auto-measuring mode. The laser diffraction particle size analysis data were evaluated using volume distribution to detect even a few large particles. The PI is a measure of the distribution of nanoparticle population (Koppel, 1972). Small PI means the narrow distribution of nanoparticles.

The zeta potential of lipid nanoparticles was determined by measurement of electrophoretic mobility using electrophoretic light scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan). The electrophoretic mobility was measured after dilution of samples with filtered deionized water at room temperature.

2.4. Incorporation efficiency

The incorporation efficiency of ITZ in lipid nanoparticles was determined by measuring the concentration of ITZ after centrifugation at 3000 × *g* for 15 min. Prior to centrifugation, ITZ-incorporated lipid nanoparticles were filtered through 1 μm membrane filter to remove free ITZ. Unprecipitated fraction of lipid nanoparticles was dissolved in methanol and diluted in an appropriate concentration for HPLC analysis. The amount of ITZ was directly determined by HPLC method. The HPLC system consisted of mobile phase delivery pump (LC-10AT, Shimadzu, Japan), UV detector (SPD-10AV, Shimadzu), autosampler (O NJ CTOR, Shimadzu) and system controller (SCL-10A, Shimadzu). Analytical column was Capcell Pak UG120 (C_{18}, 5 μm, 4.6 mm × 150 mm, Shiseido, Japan). The eluent was mixture of acetonitrile and 0.1% formic acid (80:20, v/v). The flow-rate was set at 1.0 ml/min and the injection volume was 50 μl. The eluent was monitored at an absorption wavelength of 263 nm.

2.5. Differential scanning calorimetry (DSC)

DSC analysis was performed to characterize the physical state of the lipid core in lipid nanoparticles and incorporated ITZ. Lipid nanoparticles were lyophilized without cryoprotectant to prevent the interruption of melting transition peak of cryoprotectant. The thermal characteristics of lipid nanoparticles and ITZ were determined by a differential scanning calorimeter (DSC Q-1000, TA Instrument, UK). Aliquots weighing between 5 and 10 mg were leveled in an aluminum pan and crimped with an aluminum lid. DSC was used to analyze the samples from 10 to 190 °C with a heating rate of 10 °C/min under a nitrogen flow of 20 ml/min. Indium (99.98%, melting point 156.65 °C, Aldrich, Milwaukee, USA) was used as standard for calibrating the temperature.

2.6. Powder X-ray diffractometry (PXRD)

Powder X-ray diffraction patterns were determined for ITZ and lipid nanoparticles. Lipid nanoparticles were lyophilized without cryoprotectant to prevent the interruption of crystalline peak of cryoprotectant. The powder X-ray diffraction pattern was obtained using a powder X-ray diffractometer (D5005, Bruker, Germany). The operation was performed with a voltage of 40 kV and a current of 40 mA in the region of $3^\circ \leq 2\theta \leq 70^\circ$ in step scan mode of 0.02° per second.

2.7. In vitro release test

The release test was performed with Franz diffusion cells (0.9 in diameter, Crown Scientific, Sommerville, USA) at 37 °C. Phosphate buffer saline (pH 7.4) was used as receptor fluid. There was dialysis membrane with molecular cutoff value of 3500 between upper and lower chambers. Lipid nanoparticles were diluted by addition of appropriate volume of PBS. One milliliter of diluted lipid nanoparticles containing 10 mg of ITZ was applied to upper donor chamber. A volume (24–26 ml) of PBS was added to lower receiver chamber. The solution in receiver chamber was mixed by stirring with star-shaped magnetic bar at 700 rpm. The joint between upper and lower chamber was sealed and clamped tightly to avoid fluid loss. The diffusion cells were maintained at 37 ± 5 °C using a water circulator (Refrigerated Bath Circulator, model RBC-10, Jeio Co., Korea). After collection of sample solution (100 μl), the same volume of fresh PBS was added to maintain the total volume in receiver chamber. Samples were collected over 48 h. The percentage of released ITZ was calculated by determination of the amount of ITZ in receiver medium. The concentration of ITZ in receiver medium was determined by the HPLC method.

2.8. Stability studies

Stability of lipid nanoparticles was determined by observing the changes of particle size, polydispersity index, zeta potential and incorporation efficiency during storage. Lipid nanoparticles were stored for 90 days at room temperature.

2.9. Animal studies

The healthy male Sprague–Dawley (SD) rats were purchased from Seoul National University Laboratory Animal Center (Seoul, Korea). The male SD rats weighing 300 ± 20 g were used. All experiments were performed according to the Seoul National University guidelines of experimental animal care. The experimental protocol was approved by the Institute of Laboratory Animal Resources, Seoul National University. Animals was grown in Animal Center for Pharmaceutical Research (College of Pharmacy, Seoul National University) under the condition of 20–26 °C, 40–60% RH, 13–16 times of ventilation every hour, 150–300 Lux, less than 60 db, 13–18 cm/s of air flow, 12-light and 12-dark cycle. They received commercial rodent chow (Samyang Co., Seoul, Korea) and water *ad libitum*, and were kept under standard pathogen-free conditions.

The male SD rats were fasted for 12 h before parenteral administration of formulation. The femoral artery and vein were catheterized with polyethylene tube (SP45, i.d. 0.58 mm, o.d. 0.96 mm, Natume Co., Japan) under diethyl ether anesthesia. The cannula was flushed with 0.3 ml of 0.5% heparin (25,000 IU) saline normal solution to prevent the blood clotting. Each rat was allowed to recover from anesthetization before study. Lipid nanoparticles were diluted with 2–10 volume of normal saline for injection, respectively. The formulations were slowly injected into femoral vein for about 1 min at a 5 mg/kg dose of ITZ. At 1, 5, 15, 30 min and 1, 2, 4, 6, 8, 12 h post-injection, 0.4 ml of blood was collected into heparinized polypropylene tube via femoral artery and centrifuged at 10,000 × *g* for 10 min to obtain plasma. Plasma was stored at −70 °C prior to analysis.

2.10. HPLC–MS analysis of ITZ for pharmacokinetic studies

HPLC–MS analysis was performed to determine ITZ concentration in rat heparinized plasma (Yao et al., 2001). Plasma sample (100 μl) was transferred into microcentrifuge tube and spiked with 20 μl of miconazole working solution of 10 μg/ml as an internal standard (IS). After vortex mixing for 15 s, 200 μl of acetonitrile was added. The sample was again vortex mixed for 1 min. After centrifugation at 10,000 × *g* at 4 °C for 10 min, the supernatant was transferred into an autosampler vial and a 10 μl aliquot was injected into the HPLC–MS instrument. The supernatant of high plasma concentration was diluted with mobile phase.

The HPLC system consisted of an Alliance Type 2690 separation pump, an autosampler and a column heater module from Waters (Milford, MA, USA). Chromatography was performed on a XTerra™ MS (C_{18}, 3.5 μm, 2.1 mm × 50 mm, Waters) in combination with a XTerra™ guard column (C_{18}, 5.0 μm, 3.9 mm × 20 mm, Waters). The column and autosampler temperature were maintained at 35 °C and 4 °C, respectively. The eluent was composed of acetonitrile and 10 mM ammonium formate (pH 3.8, adjusted by formic acid) (70:30, v/v). The flow-rate was set at 0.3 ml/min and the injection volume was 10 μl. The column eluent was analyzed on a ZQ (Micromass, Manchester, UK) mass spectrometer. Instrument control and data analysis was accomplished using the Mass Lynx NT Ver. 3.5 software supplied with the mass spectrometer. Analysis was performed with electrospray ionization (ESI) in the positive mode. The instrument was tuned by direct infusion of 1 μg/ml of ITZ solution. The following tune parameters were used for both ITZ and IS: capillary voltage, 3.0 kV; cone voltage, 52 V; source temperature, 150 °C; desolvation temperature, 200 °C; cone gas flow-rate, 50 l/h; desolvation gas flow-rate, 300 l/h; resolution of LM, 19.1; resolution HM 12.7; ion energy 1.7; multiplier 950; Pirani pressure of the nitrogen gas used for collision, 1×10^{-4} mbar. Selected ion monitoring (SIM) was accomplished with the ion at m/z of 705.5 for ITZ and 417.1 for IS as protonated molecular ions, respectively.

2.11. Pharmacokinetic data analysis

The following pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin™ (Scientific Consulting, Inc., NC, USA). Area under the concentration–time curve up to time infinity ($AUC_{0\rightarrow\infty}$), area under the first moment of the concentration-time curve up to time infinity ($AUMC_{0\rightarrow\infty}$), mean residence time (MRT), volume of distribution at steady state (V_{ss}), total body clearance (CL) and terminal half life ($t_{1/2}$) were cal-

Table 2
Physicochemical properties of the investigated lipid nanoparticles.

Formulation	Mean diameter (nm)	Polydispersity index	Zeta potential (mV)	Incorporation efficiency (%)
F0	224.1 ± 13.0	0.196 ± 0.016	−27.24 ± 3.31	93.8 ± 2.4
F10	192.7 ± 14.4	0.184 ± 0.015	−30.21 ± 6.43	95.2 ± 1.7
F20	222.5 ± 14.2	0.197 ± 0.018	−27.26 ± 2.65	90.4 ± 2.9
F50	241.9 ± 13.5	0.208 ± 0.019	−30.44 ± 2.87	83.0 ± 1.3

Data represent mean ± S.D. ($n = 3$).

culated. The data from different formulations were compared for statistical significance by one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Physicochemical properties of liquid–solid lipid nanoparticles

Physicochemical properties of lipid nanoparticle were investigated in terms of incorporation efficiency, particle size, PI and zeta potential (Table 2). In preliminary studies, lipid nanoparticles were prepared by using tricaprin, trilaurin, trimyristin, tripalmitin or tristearin as solid lipids. Of formulations, lipid nanoparticles containing tristearin as a solid lipid showed small PI below 0.2 until ITZ was incorporated up to 50 mg/g and higher incorporation efficiency than any other solid lipids (data not shown). In this study, lipid nanoparticles were prepared with lipid core:eggPC:Tween 80:DSPE-PEG_{2000} (100:75:15:10, mg/g). The content of triolein in the lipid core was varied as 0, 10, 20 and 50 mg/g. F10 containing 10 mg/g of triolein in lipid core showed the highest incorporation efficiency and the smallest particles sized and PI among all four formulations. Addition of large amount of liquid lipid in lipid core (F50) decreased the incorporation efficiency of lipid nanoparticles. Low incorporation efficiency of lipid nanoparticles containing high concentration of liquid lipid is due to a loss of immobilization capacity of solid lipid that could incorporate drug into lipid core (Siekmann and Westesen, 1992). Lipid nanoparticles containing large amount of triolein could not incorporate higher amount of ITZ, thus free ITZ might be expelled from lipid nanoparticles during cooling process. Consequently, free ITZ might result in loss of surface charge and agglomeration of lipid nanoparticles leading to large particle size and PI.

The zeta potentials of ITZ lipid nanoparticles were about −30 mV as shown in Table 2. Zeta potential is an electrostatic value by surface electrostatic double layer and provides an indication of the potential stability of the colloidal system. Particle aggregation is less likely to occur for charged particles due to electric repulsion (Mehnert and Mäder, 2001). High zeta potential above 30 mV (in absolute value) is known to prevent nanoparticles from aggregation, ensuring stability of lipid nanoparticles (Müller and Heinemann, 1992).

3.2. Differential scanning calorimetry (DSC)

DSC was used to investigate the existing form of ITZ in binary lipid nanoparticles. As shown in Fig. 1a, the melting endothermic peak of ITZ was observed at 166 °C, while the thermograms of the lyophilized ITZ-incorporated lipid nanoparticles did not show the endothermic peak for ITZ. This suggests that ITZ was not in crystalline state but in amorphous state. Melting point depression of solid lipid was also observed in ITZ-incorporated lipid nanoparticles. Endothermic peak of tristearin used as solid lipid was observed at 72 °C. The endothermic peak of tristearin was reduced as the amount of liquid lipid in lipid core increased. Moreover, melting point of tristearin was slightly depressed by addition of liquid lipid in lipid core. Reduction and depression of endothermic peak at high concentration of liquid lipid indicates that crystallinity of lipid core is weakened (Jenning et al., 2000). A small endothermic peak observed at 45 °C is likely attributed to DSPE-PEG_{2000}.

3.3. Powder X-ray diffractometry (PXRD)

Powder X-ray diffractometry (PXRD) is one of the most common technique used to identify crystalline structure of bulk materials (Venkateswarlu and Manjunath, 2004). Thus, PXRD was used to determine the crystalline or non-crystalline nature of ITZ in

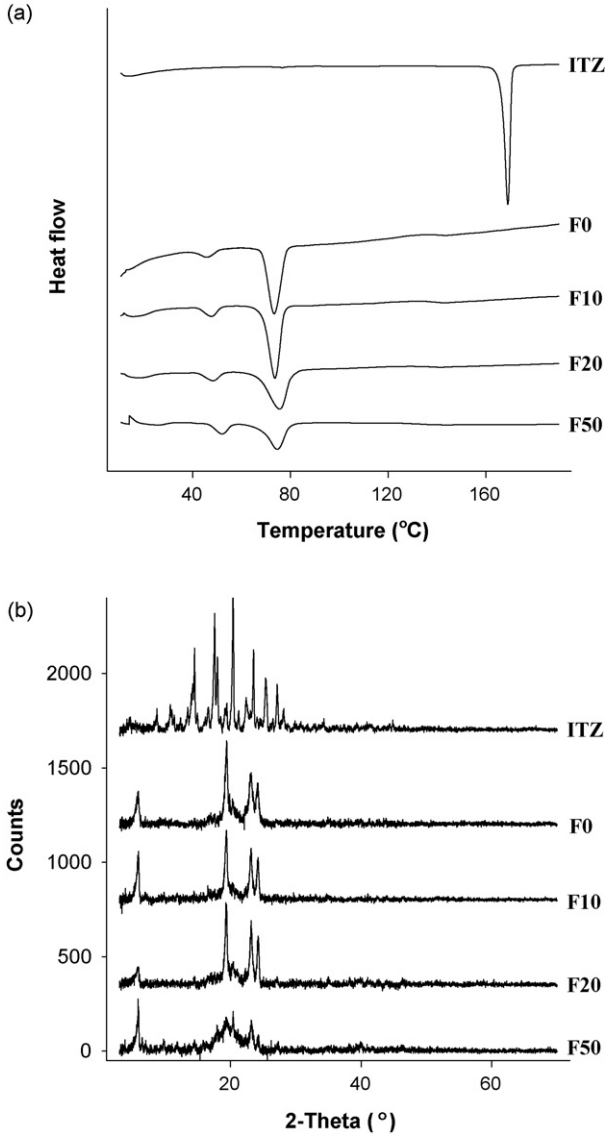

Fig. 1. DSC curves (a) and X-ray diffraction patterns (b) of ITZ and lipid nanoparticles. The thermal changes were obtained using a differential scanning calorimeter (DSC Q-1000, TA Instrument) with a heating rate of 10 °C/min. The PXRD patterns were obtained using a powder X-ray diffractometer (D5005, Bruker) in the region of $3^\circ \leq 2\theta \leq 70^\circ$ in step scan mode of 0.02° per second.

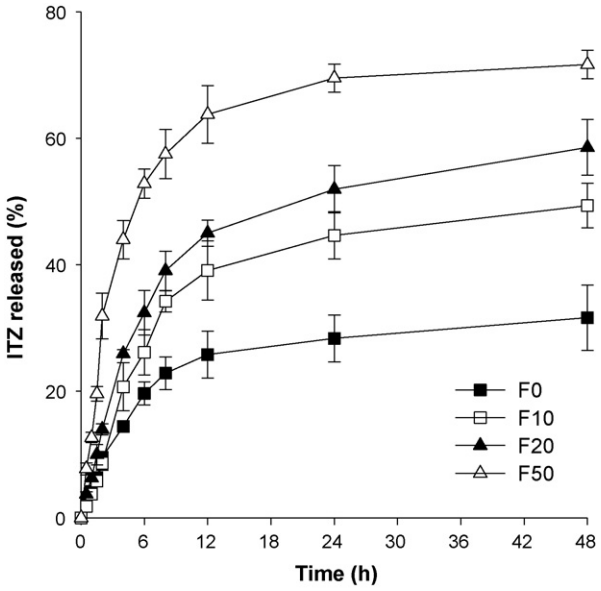

Fig. 2. Release profiles of ITZ from lipid nanoparticles in PBS (pH 7.4) at 37 °C. Data are expressed as the mean ± S.D. ($n = 3$).

the lyophilized nanoparticles powder. Lipid nanoparticles were lyophilized without cryoprotectant to prevent the interruption of crystalline peak of cryoprotectant. As shown in Fig. 1b, ITZ powder showed strong typical peaks of crystalline ITZ (Chen et al., 2008) at 2θ scattered angles 14.5°, 17.6°, 20.4°, 23.5° and 25.4°. The presence of sharp peaks indicates crystalline nature of ITZ. While there were no characteristic peaks for ITZ in lyophilized lipid nanoparticles and appeared identical to ITZ free lipid nanoparticles (F0), indicating ITZ was at an amorphous state in the ITZ-incorporated lipid nanoparticles.

3.4. In vitro release

In order to evaluate the controlled release potential of lipid nanoparticles for parenteral use, the release of ITZ from lipid nanoparticles was investigated over 48 h. Release rate of ITZ from lipid nanoparticles was significantly changed by addition of liquid lipid in lipid core (Fig. 2). The cumulative release of ITZ from lipid nanoparticles up to 48 h was in the following order: F0 (32%) < F10 (49%) < F20 (59%) < F50 (72%). The release rate of ITZ depended on the amount of liquid lipid in lipid core (Jenning et al., 2000). The release of ITZ from F50 was the fastest among the lipid nanoparticles. F0 showed the slowest release of ITZ. Probably the lipid core of solid lipid nanoparticles has less mobility than that of liquid–solid lipid nanoparticles. From the results, it is speculated that fast release rate of ITZ was induced by the weak crystallinity of liquid–solid lipid nanoparticles. The release of ITZ from lipid nanoparticles could be controlled by modulation of the amount of liquid lipid in lipid core.

3.5. Stability of lipid nanoparticles

Stability test of lipid nanoparticles was performed in terms of particle size, PI, zeta potential and incorporation efficiency during storage. F10 and F20 lipid nanoparticles were stable in particle size and PI for 90 days among those lipid nanoparticles (Fig. 3a). F0 prepared with only solid lipid and F50 containing large amount of liquid lipid showed larger particle size and PI after 90 days of storage; that is, particle size and PI of F0 and F50 increased up to 324.1 nm, 0.265 and 310.1 nm, 0.248, respectively. Both lipid nanoparticles of F0 and F50 were out of particle size and PI range for

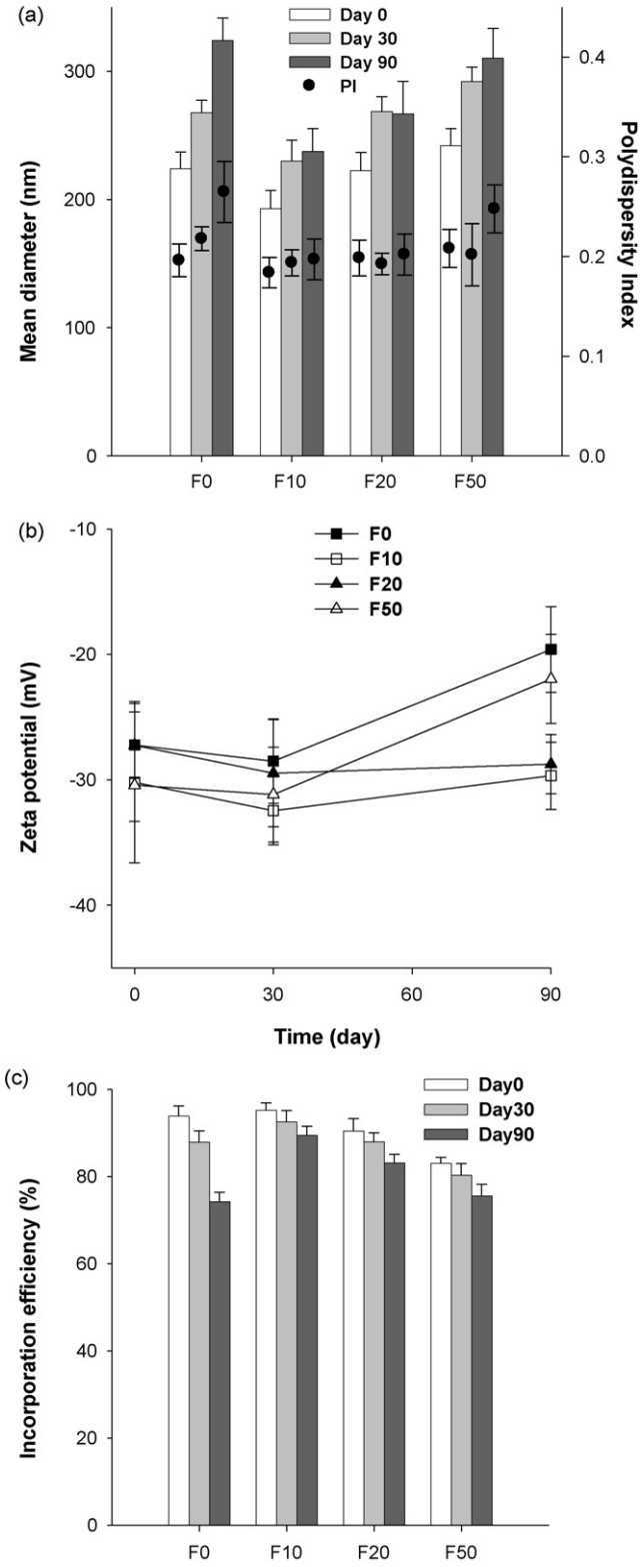

Fig. 3. The stability of lipid nanoparticles in the particle size and polydispersity index (a), zeta potential (b) and incorporation efficiency (c) during storage at room temperature. Data are expressed as the mean ± S.D. ($n = 3$).

parenteral administration after 90 days of storage. However, other lipid nanoparticles of F10 and F20 were stable in terms of particles size and PI for 30 days at room temperature.

Zeta potential changes are shown in Fig. 3b. Zeta potential was not changed in all lipid nanoparticles for 30 days at room temperature. While zeta potentials of F10 and F20 were maintained around −30 mV for 90 days, those of F0 and F50 were decreased to approximately −20 mV. Decrease of zeta potential (in absolute value) was not enough for sufficient electrostatic stabilization. There was a correlation between particle size and zeta potential.

In order to evaluate the chemical stability, drug contents in binary lipid nanoparticles were determined as literatures (Verreck et al., 2003; Hong et al., 2006). The incorporation efficiency of lipid nanoparticles prepared with solid lipid alone (F0) was drastically decreased during storage (Fig. 3c). These may be due to the recrystallization of solid lipid during storage. F10 and F20 showed above 80% of incorporation efficiency after 90 days of storage. These results indicate that the recrystallization of solid lipid could be slowed down by addition of liquid lipid in lipid core (Souto et al., 2004). However, the addition of larger amount of liquid lipid (>2%) decreased the incorporation capacity of lipid core and caused low incorporation efficiency due to limitation in drug loading. These results suggest that expulsed ITZ during storage destabilized lipid nanoparticles and changed the particle size, PI and zeta potential of lipid nanoparticles.

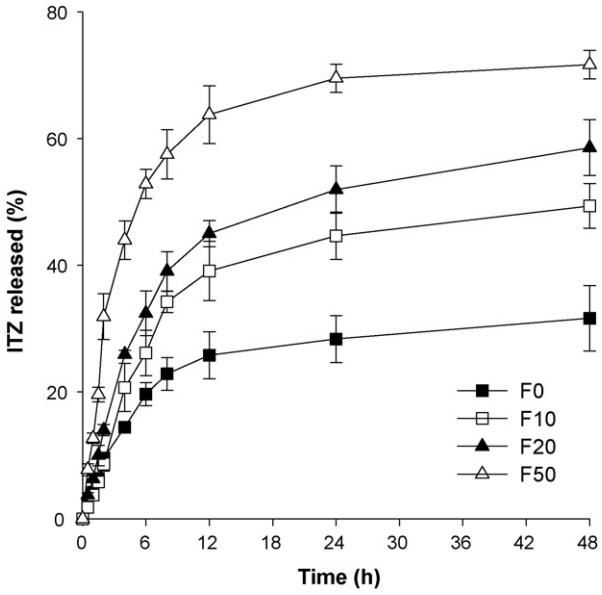

Fig. 4. Average plasma concentration versus time curves of ITZ in rats after parenteral administration of 5 mg/kg dose of ITZ in lipid nanoparticles. Data are expressed as the mean ± S.D. (n = 5).

3.6. Pharmacokinetics

The average plasma concentration vs. time curves and pharmacokinetic parameters after intravenous administration of ITZ lipid nanoparticles to SD rats (5.0 mg/kg) were compared. In previous reports, higher doses of ITZ (10, 20, or 30 mg/kg) were intravenously administered to rats (Miyazaki et al., 1993; Shin et al., 2004). However, since the sustained release of ITZ from binary lipid nanoparticles was expected, we reduced drug dose as 5 mg/kg. As shown in Fig. 4, F50 displayed rapid clearance from blood plasma, being consistent with *in vitro* release pattern of formulation. The prolonged circulation in blood plasma was observed in F10 and F20 lipid nanoparticles. F0 prepared with solid lipid alone as lipid core showed the longest circulation in blood plasma. The $t_{1/2}$ of ITZ in F0 was comparable to that of ITZ-loaded polymeric micellar formulation (Yi et al., 2007). $AUC_{0\to\infty}$ of ITZ in lipid nanoparticles except F50 increased significantly (Table 3). F50 shows no differences in pharmacokinetic parameters compared with the reported data of Sporanox® (5 mg/kg) (Rabinow et al., 2007). However, F0 and F10 showed significant difference compared with F50 in $AUMC_{0\to\infty}$, MRT, CL and $t_{1/2}$ of formulation as shown in Table 3. The difference among lipid nanoparticles appears to be related to the amount of liquid lipid in lipid core. These data suggest that lipid nanoparticles containing larger amount of liquid lipid in lipid core are rapidly degraded by enzymes present in blood and are cleared in blood plasma quickly. If the concentration of DSPE-PEG_{2000} was variable, significantly different plasma concentration would be obtained. Moreover, DSPE-PEG_{2000}, used as a steric stabilizer in our formulation probably contributes to the prolonged circulation of lipid nanoparticles (Stevens et al., 2004). In regards to DSPE-PEG_{2000}, it was also reported that eggPC alone was not sufficient to stabilize the lipid nanoparticles; PEGylated phospholipids (DSPE-PEG_{2000}) were added to decrease gel formation during preparation and storage of lipid nanoparticles and increase stabilization by steric hindrance (Lee et al., 2007). Additionally, PEGylated phospholipids have a benefit of potentially increasing the circulation time of nanoparticles in the body (Kim et al., 2009). In further study, we could try to investigate the effect of DSPE-PEG_{2000} in pharmacokinetic study.

Although binary lipid nanoparticles could be also clinically applied for parenteral nutrition and several drug loaded formulation, the lipid-based formulations have a limit to incorporate a small quantity of drug. Thus, nanosuspension could be as an alternative with feasibility to formulate compounds insoluble in both water and oils (Constantinides et al., 2008). In particular, since the solubilization of high melting point compounds is difficult in any solvent, nanosuspensions can be used to maintain these drugs in a preferred crystalline state of sufficiently small size for intravenous administration. However, our results with binary lipid nanoparticles of ITZ (5 mg/kg) are comparable to those with nanosuspension of ITZ (20 mg/kg) (Rabinow et al., 2007), although it is difficult to compare directly due to the difference in doses. Furthermore, oral fenofibrate nanosuspension and SLN did not show

Table 3
Non-compartmental pharmacokinetic parameters after parenteral administration of 5 mg/kg dose of ITZ in lipid nanoparticles to rats (n = 5).

Parameters	Formulation			
	F0	F10	F20	F50
$AUC_{0\to\infty}$ (μg h/mL)	25.5 ± 5.8[a,b]	20.7 ± 4.8[a]	17.2 ± 3.5[a]	8.6 ± 1.3
$AUMC_{0\to\infty}$ (μg h^2/ml)	262.5 ± 146.6[a,b]	171.1 ± 73.4[a]	131.4 ± 64.9	33.1 ± 11.9
MRT (h)	10.0 ± 3.5[a,b]	8.0 ± 2.2[a]	7.0 ± 2.4[a]	3.8 ± 0.8
V_{ss} (l/kg)	2.0 ± 0.7	1.9 ± 0.4	2.2 ± 0.6	2.2 ± 0.4
CL (l/h/kg)	0.2 ± 0.1[a,b]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.6 ± 0.1
$t_{1/2}$ (h)	7.3 ± 2.9[a]	6.0 ± 1.6[a]	5.8 ± 2.2[a]	2.8 ± 0.9

[a] Significantly different from F50 (p < 0.05).
[b] Significantly different from F20 (p < 0.05).

significant difference in pharmacokinetic evaluation (Hanafy et al., 2007).

4. Conclusion

In the present work, injectable ITZ-loaded binary lipid nanoparticles were successfully prepared by high-pressure homogenization. These binary lipid nanoparticles contained an established composition of triolein as the lipid core and tristearin as the solid core, along with PEGylated phospholipid as stabilizer. The release of ITZ from lipid nanoparticles was accelerated by increasing the amount of liquid lipid in lipid core. Moreover, the plasma concentration of ITZ was prolonged after intravenous administration of lipid nanoparticles. These results suggest that ITZ-loaded binary lipid nanoparticles have promising potential as alternative parenteral formulation of ITZ. Further studies are needed to apply the oral administration of ITZ binary lipid nanoparticles and to investigate the altered pharmacokinetic parameters.

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