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Research paper

Increasing the oral bioavailability of the poorly water soluble drug itraconazole with ordered mesoporous silica

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Abstract

This study aims to evaluate the *in vivo* performance of ordered mesoporous silica (OMS) as a carrier for poorly water soluble drugs. Itraconazole was selected as model compound. Physicochemical characterization was carried out by SEM, TEM, nitrogen adsorption, DSC, TGA and *in vitro* dissolution. After loading itraconazole into OMS, its oral bioavailability was compared with the crystalline drug and the marketed product Sporanox® in rabbits and dogs. Plasma concentrations of itraconazole and OH–itraconazole were determined by HPLC-UV. After administration of crystalline itraconazole in dogs (20 mg), no systemic itraconazole could be detected. Using OMS as a carrier, the AUC_{0-8} was boosted to 681 ± 566 nM h. In rabbits, the AUC_{0-24} increased significantly from 521 ± 159 nM h after oral administration of crystalline itraconazole (8 mg) to 1069 ± 278 nM h when this dose was loaded into OMS. T_{max} decreased from 9.8 ± 1.8 to 4.2 ± 1.8 h. No significant differences (AUC, C_{max}, and T_{max}) could be determined when comparing OMS with Sporanox® in both species. The oral bioavailability of itraconazole formulated with OMS as a carrier compares well with the marketed product Sporanox®, in rabbits as well as in dogs. OMS can therefore be considered as a promising carrier to achieve enhanced oral bioavailability for drugs with extremely low water solubility.

Keywords: Ordered mesoporous silica; Enhanced dissolution; Poorly water soluble drugs; Oral bioavailability; Itraconazole

1. Introduction

The majority of innovative drugs emerging from drug discovery programs display very poor water solubility characteristics, resulting in poor oral bioavailability due to insufficient dissolution throughout the gastrointestinal tract [1]. Developing strategies to overcome this handicap and to enable oral delivery of these new chemical entities now constitutes one of the greatest challenges to scientists active in pharmaceutical research [2]. Although several formulation approaches including solid dispersions [3], emulsion based systems [4] and nanosizing [5] have led to promising *in vitro* results, the number of marketed applications of these technologies remains very limited. Together with the growing number of poorly water soluble compounds, this emphasizes the need to explore new types of approaches.

Recently, ordered mesoporous (2 nm < pore size < 50 nm) silica materials ($SiO_2 \cdot xH_2O$), designated OMS, have attracted much attention because of their emerging applications in drug delivery [6]. Since their first appearance in materials science in the 1990s, these inorganic carriers have been successfully used in other research

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areas such as catalysis [7] and adsorption [8]. The majority of ordered mesoporous materials have a two dimensionally ordered array of cylindrical pores of uniform size disposed parallel to each other and separated by thin walls [9]. MCM-41 (Mobil Composition of Matter number forty one) and SBA-15 (Santa Barbara Amorphous number fifteen) are probably the most investigated materials of this type. SBA-15 was first described by Zhao et al. and is the result of a templating procedure based on a hexagonal arrangement of amphiphilic block copolymers [10]. MCM-41 is obtained by the template action of long chain alkylammonium surfactant molecules [9]. Typically, the pore diameter varies between 2 and 6 nm for MCM-41 and between 4 and 13 nm for SBA-15. In addition to the well-defined mesopore system, SBA-15 has a complementary pore system comprised of micropores (pore size < 2 nm). These micropores are located in the walls between adjacent mesopores and do not bridge the wall; they constitute dead end pores [12]. Due to their open and well-defined pore system in combination with high surface area, ordered mesoporous silica materials are potential carriers for therapeutic molecules [13]. Vallet-Regi et al. were one of the first to explore the drug release properties of these materials in an attempt to prolong the release of ibuprofen using MCM-41 as a carrier [14]. The release kinetics of drugs from OMS carriers is dependent on several material characteristics including pore size [15], pore connectivity [16] and the chemical composition of the silica surface [17]. Initially, the focus of mesoporous silica materials has been on the development of slow release formulations; but the applicability has recently been expanded towards dissolution enhancement of poorly water soluble compounds. Charnay et al. showed a rapid and pH dependent release of ibuprofen from MCM-41 with a pore size of 3.5 nm [18]. Later studies evidenced a high mobility of ibuprofen inside the pore system of MCM-41 at ambient temperature and pointed out the weak interaction of ibuprofen with the silica matrix. Although this NMR study was performed with loaded MCM-41 in the dried state, this behavior was suggested to be favorable to obtain rapid release kinetics of ibuprofen [19]. In a more recent paper dealing with the foam-like mesoporous silica structure TUD-1 (Technische Universiteit Delft number one), the uptake and fast *in vitro* release of ibuprofen was demonstrated and ascribed to the presence of a three-dimensional mesopore system [20].

Our group recently reported on the use of OMS and demonstrated that the presence of a sufficiently wide pore diameter is the key for accelerating the release of the poorly soluble drug itraconazole [21]. This anti-fungal triazole compound has an estimated aqueous solubility of ca. 1 ng ml^{-1} at neutral pH and ca. 4 μg ml^{-1} at pH 1 [22]. Due to its high lattice energy and extremely hydrophobic character, itraconazole is a good model compound for low-solubility drugs in order to evaluate the ability of OMS materials to improve the dissolution properties. The *in vitro* performance of silica based materials has been demonstrated in various studies. To the best of our knowledge, the biopharmaceutical performance of these novel materials has not been documented so far.

In the present study, the *in vivo* performance of OMS based formulations was determined in rabbits and dogs by comparing the bioavailibility of itraconazole loaded into OMS with the marketed product Sporanox® and pure crystalline itraconazole. The oral bioavailability of itraconazole formulated with OMS as a carrier compares well with the marketed product Sporanox®, in rabbits as well as in dogs. These results evidence that OMS is a promising carrier to achieve enhanced oral bioavailability for drugs with poor aqueous solubility.

2. Materials and methods

2.1. Ordered mesoporous silica synthesis

Ordered mesoporous silica (OMS) was synthesized according to the procedure described by Kosuge et al. [11]. Briefly, 6 g of triblock copolymer Pluronic P123 (BTC-Benelux, La Hulpe, Belgium) was dissolved in 180 g of 2 M HCl. This mixture was placed in an oil bath at 35 °C under magnetic stirring. An amount of 15.3 g of sodium silicate solution (>27 wt.-% SiO_2, Riedel-de Haën, Seelze, Germany) was diluted with 45 g demineralized water. This mixture was added dropwise to the Pluronic P123 solution under vigorous stirring. The stirring was allowed to continue for another 5 min before switching to static synthesis conditions at 35 °C. After 24 h, the silica suspension was transferred into a teflon lined autoclave (K.U. Leuven workshop) and placed in an oven for hydrothermal treatment at a temperature of 90 °C for another 48 h. Finally, the powder was washed on a 0.45 μm filter (Whatman Schleicher and Schuell, Dassel, Germany) with demineralized water, dried and calcined at 550 °C for 8 h under ambient atmosphere to remove the triblock copolymer from the pores.

2.2. Itraconazole loading procedure

Loading of OMS was performed by suspending the powder into an itraconazole (Janssen Pharmaceutica, Beerse, Belgium) solution in methylene chloride (5 mg ml^{-1}). The mixture was agitated for 24 h using a rotary mixer (20 rpm, Snijders, Tilburg, The Netherlands). The initial OMS and itraconazole weight ratio amounted to 75 wt.-% and 25 wt.-%, respectively. Subsequently, the solvent was removed by evaporation and the powder was dried overnight at 35 °C. OMS loaded with itraconazole was heated to 100 °C for 5 min and placed under reduced pressure (10^{-3} bar) at 40 °C for another 48 h to ensure the complete removal of methylene chloride. The final itraconazole loading was determined using a long-term release experiment during seven days under sink conditions (0.1 M HCl, 0.5 wt.-% SLS, $n = 5$). This value was verified with

thermogravimetric analysis (TGA, TA Instruments, Brussels, Belgium).

2.3. Nitrogen adsorption and calculations

Nitrogen adsorption isotherms were obtained using a Micromeritics Tristar 3000 (Micromeritics Instrument Corp., Norcross, USA). The measurements were performed at −196 °C and all samples were pre-treated at 40 °C for 12 h under nitrogen flushing prior to analysis. The total surface area is computed with the BET model in the relative pressure range between 0.05 and 0.2. The total pore volume was assessed using the *t*-plot method of de Boer [23]. The *t*-plot expresses the volume of nitrogen ($cm^3\ g^{-1}$) adsorbed onto the silica surface as a function of the statistical film thickness (nm) of nitrogen, adsorbed on a non-porous reference surface. The mesopore sizes (nm) were computed from the adsorption branches of the nitrogen isotherms using the Barrett–Joyner–Halenda (BJH) approach. This model is based on the Kelvin equation to describe the pore filling through capillary condensation [24].

2.4. Scanning electron microscopy and transmission electron microscopy

Scanning electron microscopy (SEM) was undertaken with a Philips SEM XL30 FEG instrument (Philips, Eindhoven, The Netherlands). The samples were gold-plated prior to imaging. Transmission electron micrographs (TEM) were obtained using a Philips CM20 (Philips, Eindhoven, The Netherlands) operated at 200 kV. Before examination, the silica material was dispersed in ethanol and deposited on a copper grid. Pictures presented are representative for the whole sample.

2.5. Differential scanning calorimetry

In order to study the physical state of itraconazole in the mesoporous silica matrix, the loaded powders were analyzed using a DSC Q1000 (TA Instruments, Brussels, Belgium). The sample was heated from 20 to 200 °C at 30 °C min^{-1}. Indium was used to calibrate the temperature scale and the enthalpic response. The samples (weight range 6–10 mg) were analyzed in open aluminum sample pans (TA Instruments, Brussels, Belgium). An amount of 0.1 mg of crystalline or glassy itraconazole was still readily detectable with this procedure.

2.6. Thermogravimetric analysis

TGA was performed on a TGA Q500 (TA Instruments, Brussels, Belgium) under N_2:O_2 (90:10) flow. The samples were heated to 150 °C to remove the physically adsorbed water. After 30 min at 150 °C, the temperature was raised to 800 °C at 5 °C min^{-1}. The itraconazole content was determined from the weight loss between 200 and 800 °C together with a correction for the silanol condensation and chemical water evacuation from the surfaces determined from the weight loss recorded on unloaded OMS.

2.7. In vitro dissolution study of OMS loaded with itraconazole and pure crystalline itraconazole

In order to study release of itraconazole from its OMS carrier, the loaded powders were suspended in simulated gastric fluid (0.1 M HCl containing 0.2 wt.-% NaCl) in the absence or presence of 0.5 wt.-% sodium lauryl sulfate (Certa s.a., Braine-l'Alleud, Belgium). SLS was added when sink conditions were preferred. The dissolution study was performed in test tubes of 10 ml under gentle agitation using a rotary mixer (Snijders-Tilburg, Tilburg, The Netherlands). The amount of material in the dissolution medium was adjusted to obtain a fixed concentration of drug substance (0.08 mg ml^{-1}). This corresponds to approximately 10% of the saturation solubility of itraconazole in simulated gastric fluid containing 0.5 wt.-% SLS. At specific time intervals, samples were collected and the medium was filtered through a 0.45 μm PTFE membrane. Prior to analysis by HPLC, samples were diluted with methanol (1:1) to prevent precipitation during analysis.

2.8. HPLC assay

Samples of the *in vitro* dissolution study were assayed using an isocratic HPLC method. The HPLC system consisted of a LaChrom® L-7100 HPLC pump, an autosampler model L-7200 equipped with a 100 μl loop, a UV detector model L-7420 set at 260 nm, and an Interface D-7000 (all Merck, Darmstadt, Germany). UV signals were monitored and peaks were integrated using the D-7000 HSM software. The separation of itraconazole was performed on a RP-18 150 × 4.6 mm 5 μm Hypersil silica column (Thermo Electron Corporation, Waltham, USA) at room temperature. The mobile phase consisted of acetonitrile:tetrabutyl ammonium hydrogen sulfate 0.01 N (55:45 v/v), and was filtered through a 0.45 μm PTFE membrane and degassed by ultrasonication before use. The flow rate amounted to 1.5 ml min^{-1}. The standard curves were linear over the concentration range of 0.0001–3 mg ml^{-1}.

2.9. Dosage form

Itraconazole loaded into OMS and crystalline itra-conazole were mixed with croscarmellose, lactose and SLS. The physical mixture was filled into hard gelatin capsules size 3 (rabbits) and size 00 (dogs). Croscarmellose (25 wt.-%), lactose (25 wt.-%) and SLS (1 wt.-%) were included to ensure rapid capsule disintegration [25]. Sporanox® pellets were removed from the commercial capsules and refilled into the same hard gelatin capsules. The itraconazole capsules dose amounted to 8.1 ± 0.1 mg (itraconazole loaded into OMS), 8.6 ± 0.1 mg (Sporanox®) and 8.4 ± 0.2 mg (crystalline itraconazole) for the rabbit

study and 20.4 ± 0.1 mg (itraconazole loaded into OMS), 23.2 ± 0.1 mg (Sporanox®) and 22.8 ± 0.2 mg (crystalline itraconazole) for the dog study. All results presented (AUC, C_{max}, T_{max}, and concentration–time profiles) in this study were normalized to the dose provided by the OMS capsules.

2.10. In vivo studies

2.10.1. Animal experiments

New Zealand White rabbits (4–6 months of age, 3.6–4.1 kg, female) are housed – according to the Belgian and European laws, guidelines and policies for animal experiments, housing and care – in the Central Animal Facilities of the K.U. Leuven. These facilities have the obligatory accreditation of the authorized Belgian Ministry and are registered under license number LA1210261. Approval for this project was granted by the Institutional Ethical Committee for Animal Experimentation. Prior to oral drug administration, the rabbits ($n = 5$) were fasted overnight (>12 h). After receiving the oral dose, 3 ml of water was administered to facilitate swallowing. After 4 h, the rabbits had free access to food and water. For the comparison of the formulation based on OMS loaded with itraconazole and Sporanox®, the rabbits were dosed according to a cross-over design ($n = 5$). In a subsequent experiment, all rabbits were dosed with a capsule containing pure crystalline itraconazole. A wash out period of seven days was allowed between subsequent dose administrations. Blood samples (2.5 ml) were collected from the *vena auricularis* prior to dosing and at 0.5, 1, 2, 3, 4, 7, 10, 12 and 24 h after dosing. Immediately after blood collection, plasma was harvested by centrifugation at 2500*g* for 10 min. Plasma was then transferred to a fresh Eppendorf tube and frozen at −20 °C prior to analysis.

Marshall Beagle dogs (1.5–3 years of age, 7.1–10.0 kg, male) were housed in the animal facility of J & JPRD division of Janssen Pharmaceutica (Beerse, Belgium) with free access to water and food. The comparative bioavailability study was also performed according to a cross-over design ($n = 5$). Dogs were treated in accordance with the following legislation: the provisions of the Belgian law of October 18, 1991 on the approval of the European convention on the protection of vertebrates that are used for experimental and other scientific purposes, and also of annexes A and B, drawn up in Strasbourg on March 18, 1986, the Royal Decree of November 14, 1993 on the protection of laboratory animals. After administration of the capsules, 10 ml of water was given to facilitate swallowing. The evaluation of the formulation containing pure crystalline itraconazole was performed with 4 out of 5 dogs. Blood samples (2.5 ml) were collected from the *vena jugularis* before dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 8 h after dosing. Immediately after blood collection, plasma was harvested by centrifugation at 2500*g* for 10 min. Plasma was then transferred to a fresh tube and frozen at −20 °C prior to analysis.

2.10.2. Plasma processing and analysis

Plasma concentrations of itraconazole and hydroxyitraconazole were determined by a validated HPLC method. To 1 ml of plasma, 100 μl of the internal standard solution was added [R051012 (Janssen Pharmaceutica), 2.5 μM in 0.2 M HCl]. After addition of 500 μl 2 M NaOH, itraconazole and hydroxyitraconazole were extracted with 4 ml diethyl ether. Following centrifugation at 4000 rpm for 5 min, the upper organic layer was transferred into a fresh tube. The organic solvent was evaporated under a gentle stream of air and the extraction residue was dissolved in 200 μl methanol:water mixture (50:50 v/v), of which 99.5 μl was injected into the HPLC system. Concentrations of itraconazole and hydroxyitraconazole were determined using an isocratic HPLC method. The HPLC system (Merck-Hitachi, Darmstadt, Germany) used for the analysis of the plasma samples consisted of an Elite LaCrom L-2130 HPLC pump, an autosampler model L-2200 equipped with a 100 μl loop and a UV detector model L-2400. Separations were achieved using a Novapak C-18, 4 μm, under radial compression. The mobile phase consisted of methanol:25 mM sodium acetate pH 3.3 (78:22 v/v). The mobile phase was filtered through a 0.45 μm PTFE membrane before use. The flow rate of the mobile phase was maintained at 1.75 ml min^{-1} and the effluent was monitored at a wavelength of 265 nm. Itraconazole, hydroxyitraconazole and R051012 were eluted with retention times of 4.5, 8.0 and 11.5 min, respectively. The standard curves were linear over the concentration range of 7.8–500 nM. The intraday reproducibility, expressed as the relative standard deviation, was less than 7.3% for itraconazole and less than 5.4% for hydroxyitraconazole over the dose range studied ($n = 6$).

2.10.3. Data presentation and analysis

The maximal plasma concentrations (C_{max}) and the time to reach C_{max} (T_{max}) were determined from the individual time versus concentration profiles. Systemic exposure was determined by calculating $AUC_{0-24\,h}$ (rabbits) and $AUC_{0-8\,h}$ (dogs) in Microsoft Excel® using the linear trapezoidal rule. The pharmacokinetic parameters between the different formulations were statistically compared with the non-parametric Wilcoxon signed-rank test. The level of significance was set at $p < 0.03$ for comparing two out of three groups.

3. Results and discussion

3.1. Characterization of OMS and loading with itraconazole

OMS was synthesized and characterized in terms of porosity, internal structure and morphology by electron microscopy and nitrogen adsorption. Fig. 1 displays SEM and TEM images of the OMS under investigation. The TEM image clearly shows the hexagonal arrangement of the pore system and the uniformity of the cylindrical pores. The combined distance of pore together with the

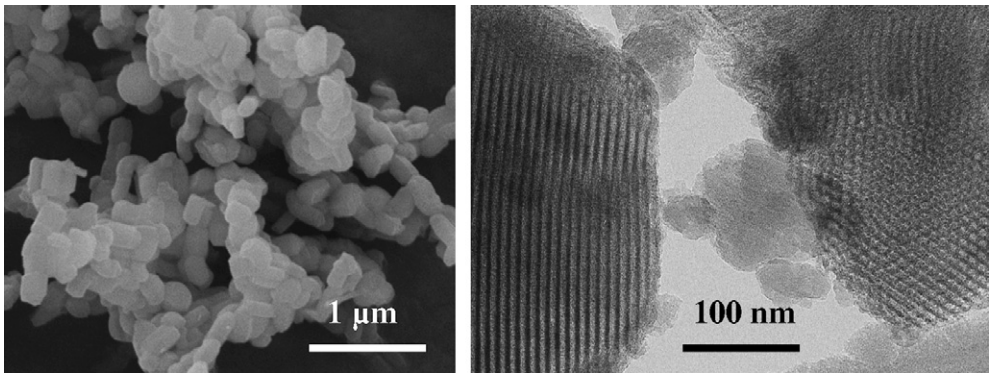

Fig. 1. SEM (left) and TEM (right) image of OMS. Scale bars correspond to 1 μm and 100 nm, respectively.

pore wall amounted to 9.2 nm. The morphology of OMS is defined as single particles with sizes ranging from 0.2 to 1 μm which act as randomly oriented building blocks to form larger aggregates of approximately 50 μm as presented by SEM analysis. The internal pore structure was determined using nitrogen adsorption. The nitrogen adsorption isotherm presented a hysteresis loop with parallel steep branches typical of material with a uniform pore size. Nitrogen adsorption isotherms of OMS, before and after loading with itraconazole, are presented in Fig. 2. Drug loading itself was determined by a long-term release under sink conditions and by TGA, resulting in a loading percentage of 20.5 ± 0.6 wt.-% and 21.3 ± 0.5 wt.-%, respectively. Loading ordered mesoporous OMS with itraconazole significantly changed the porosity of the sample due to the incorporation of itraconazole into the pores. A decrease in amount of nitrogen adsorbed reflects the decreased pore volume of the carrier, while the slightly shifted hysteresis loop to lower p/p_0 values upon loading with itraconazole characterizes a reduced pore size. The total pore volume was decreased from 0.85 to 0.50 $cm^3\ g^{-1}$ after loading OMS with itraconazole. The mesopore diameter of OMS according to nitrogen adsorption is ca. 7.3 nm. After itraconazole uptake, the mesopore diameter probed with nitrogen was decreased to 6.6 nm. Loading with itraconazole also altered the BET surface area of OMS, which decreased from 844 to 355 $m^2\ g^{-1}$.

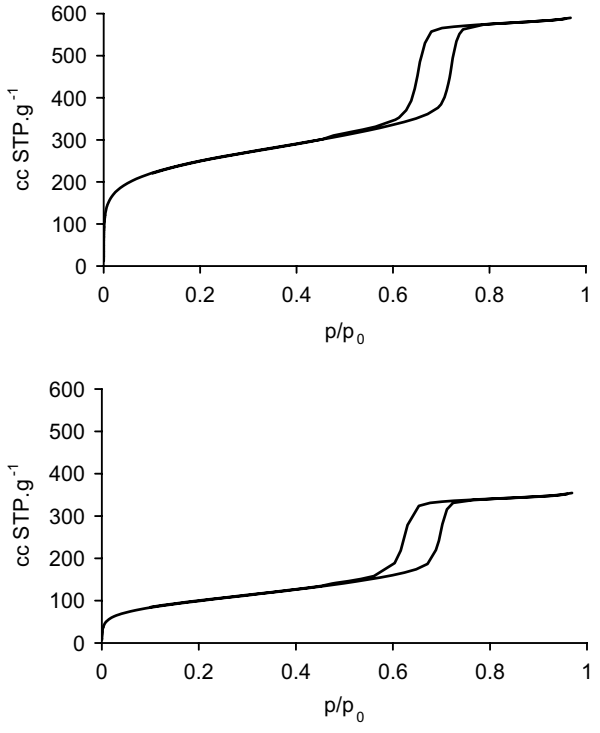

Fig. 2. Nitrogen adsorption isotherms of pure OMS (top) and after loading with itraconazole (bottom).

Previous research evidenced that, in order to maximize the dissolution rate enhancement, itraconazole molecules need to be in a molecular state when adsorbed onto OMS [21]. In the present study, the physical state of itraconazole was assessed using DSC. Crystalline itraconazole melts at 168 °C, while glassy itraconazole is characterized by a glass transition at 60 °C. These transitions allow differentiation to be made between the presence of drug clusters, either glassy or crystalline, or drug that is molecularly deposited onto the inner and outer surface of OMS. Thermograms of OMS loaded with itraconazole were recorded and signals owing to glass transition and to melting could not be observed (data not shown). The only broad endothermic event with a maximum intensity around 90 °C can be attributed to the desorption of physically adsorbed water. The absence of phase transitions owing to itraconazole in DSC analysis evidences that itraconazole is in a molecular state.

3.2. In vitro dissolution study

Release of itraconazole from OMS was compared with the dissolution of pure crystalline itraconazole in simulated gastric fluid (SGF, 0.1 M HCl) and under sink conditions created by SLS in the medium (SGF–SLS, 0.1 M HCl). The *in vitro* profiles are depicted in Fig. 3. In SGF–SLS the release of itraconazole from OMS was very fast with $76.4 \pm 2.2\%$ from the initial itraconazole content being released after 5 min. When pure crystalline itraconazole was used, only $19.0 \pm 1.5\%$ was dissolved into the medium after 5 min. However, after 1 h, the difference vanishes and equal amounts are solubilized in SGF–SLS, illustrating

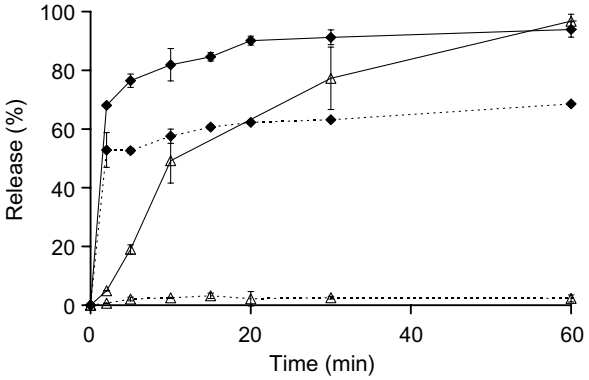

Fig. 3. Dissolution of pure crystalline itraconazole (△) and the release of itraconazole from OMS (◆) in simulated gastric fluid in the absence (dashed) and presence (solid) of SLS. All experiments were performed in duplicate (average and range depicted).

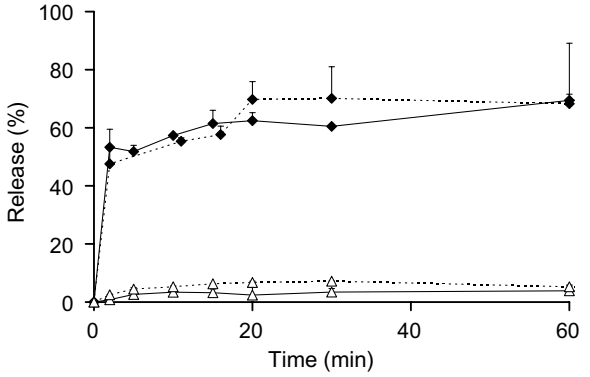

Fig. 4. Effect of the added excipients included in the hard gelatine capsules on the dissolution of crystalline itraconazole (△) and on the release of itraconazole from OMS (◆). All experiments were performed in duplicate (average and half range depicted). Dashed lines represent the case where excipients are included.

that the inclusion of SLS reduces the discriminative power of the medium. Therefore, the comparison between release of itraconazole from OMS and the dissolution of pure crystalline itraconazole was performed in SGF without SLS. The same enhanced release behavior was also manifested in non-sink dissolution medium, achieving a supersaturated solution with a 11-fold higher itraconazole concentration ($\sim$45 μg ml^{-1}) compared to its thermodynamic solubility at pH 1 ($\sim$4 μg ml^{-1}). After 5 min, 52.6 ± 0.9% from the initial amount of itraconazole into OMS was released, in contrast to a dissolution of only 2.1 ± 0.6% in the instance of pure crystalline itraconazole. These data clearly illustrate that loading itraconazole into OMS circumvents the slow dissolution kinetics and low water solubility of the pure crystalline drug in non-sink conditions. The enhanced release kinetics of itraconazole can be attributed to the molecular state of the drug.

In order to evaluate the biopharmaceutical performance of OMS as a carrier for itraconazole, hard gelatine capsules were used. To ensure rapid disintegration, OMS loaded with itraconazole (49 wt.-%) was mixed with croscarmellose (25 wt.-%), lactose (25 wt.-%) and SLS (1 wt.-%). This combination resulted in disintegration times of less than 1 min, followed by a good dispersion of the loaded OMS into the dissolution medium. The possible influence of the excipients on the release properties of the OMS loaded with itra-conazole or on the dissolution and solubility of pure crystalline itraconazole was investigated. Fig. 4 shows a comparison of the release of itraconazole from OMS and dissolution of crystalline itraconazole with and without excipients. From this comparison, it is clear that the excipients had no influence on the dissolution or solubility of crystalline itraconazole or on the release of itraconazole from OMS.

3.3. In vivo evaluation of itraconazole from OMS, Sporanox® and crystalline itraconazole

Two animal species, rabbits and dogs, were selected to investigate whether the fast *in vitro* release kinetics can be translated into an increased bioavailibility of the drug, due to improved intraluminal dissolution. Three different formulations were assessed: OMS based formulations, Sporanox® and crystalline itraconazole. Fig. 5 shows the average plasma concentration versus time curves of itraconazole and the active metabolite hydroxyitraconazole after dosing itraconazole in rabbits. High intersubject variability was encountered in all three cases; wide scattering in pharmacokinetic data for itraconazole has been reported before in the literature for humans as well as laboratory animals [26]. Administration of crystalline itraconazole resulted in an AUC_{0-24} value of 521 ± 159 nM h and a

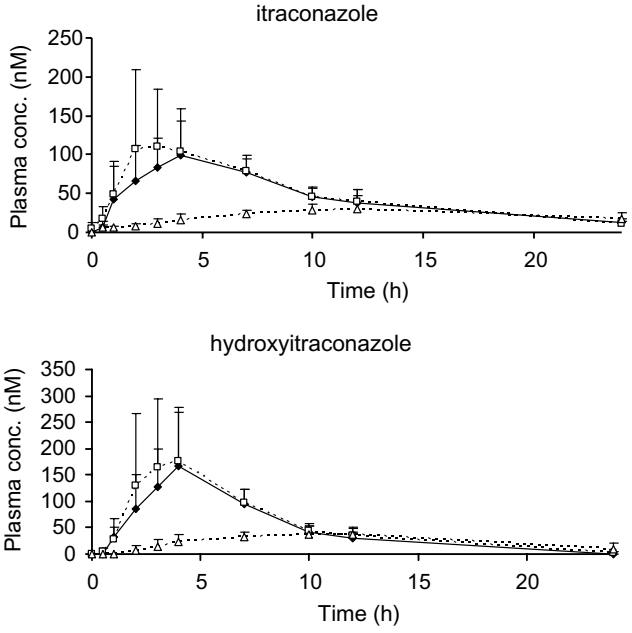

Fig. 5. Plasma concentration–time profiles of itraconazole and hydroxyitraconazole after single dosing with OMS loaded with itraconazole (◆), Sporanox® (□) and crystalline itraconazole (△) in rabbits ($n = 5$). Concentrations were dose normalized to the amount provided by the OMS capsules (8.1 ± 0.1 mg) (average + SD).

T_{max} of 9.8 ± 1.8 h. When the same dose of itraconazole was formulated into OMS, the systemic exposure to itraconazole was raised significantly as reflected in an AUC_{0-24} of 1069 ± 278 nM h. In addition, T_{max} decreased to 4.2 ± 1.8 h. These pharmacokinetic profiles of itraconazole in rabbits reflect the *in vitro* dissolution enhancement achieved when OMS is used as a carrier for itraconazole. The *in vivo* results obtained for the formulation based on OMS were compared in a cross-over study with the marketed product Sporanox®. The plasma profiles did not differ significantly; after oral dosing with Sporanox® the C_{max}, T_{max} and AUC_{0-24} corresponded to 132 ± 88 nM, 5.2 ± 2.5 h and 1155 ± 424 nM h, respectively.

The OMS formulation and Sporanox® were also evaluated in a cross-over study in 5 dogs. In this case, drug plasma levels up to 8 h are reported. This study design was too short to allow a full characterization of the bioavailability, but the results obtained support the fact that the use of OMS results in an enhanced extent of absorption. In an initial experiment, oral dosing of pure crystalline itraconazole was also assessed in 4 dogs. In contrast to rabbits, no systemic concentrations of itraconazole and its main metabolite hydroxyitraconazole could be observed after oral dosing of pure crystalline itraconazole. One may speculate that this can be attributed to a higher pH in the stomach of dogs or a shorter residence time in the acidic environment of the stomach [27,28], both impeding sufficient dissolution and thus absorption. When itraconazole is dosed to dogs as a formulation based on OMS, the oral bioavailability is boosted significantly: after administration of a 20 mg itraconazole dose, a mean C_{max} value of 135 ± 114 nM and AUC_{0-8} of 681 ± 566 nM h are recorded. Fig. 6 shows the average plasma concentration versus time curves of itraconazole and the active metabolite hydroxyitraconazole in dogs. This evidences the capability of OMS to enhance the oral bioavailibilty of itraconazole. T_{max} amounted to 1.8 ± 0.5 h which can be attributed to the shorter gastric residence in dogs compared to rabbits where higher T_{max} values were recorded. When Sporanox® was administered at the same itraconazole dose level, C_{max} values of 162 ± 86 nM and a systemic exposure expressed as AUC_{0-8} of 760 ± 364 nM h were obtained. Table 1 summarizes the AUC values of itraconazole and hydroxyitraconazole obtained with the different formulations in dogs as well as in rabbits. The plasma levels of itraconazole and its metabolite hydroxyitraconazole obtained after oral dosing of the OMS formulation were statistically not significantly different from the results obtained with Sporanox®. This comparative bioavailability study clearly demonstrates that OMS is a promising carrier to enhance the dissolution of poorly water soluble compounds.

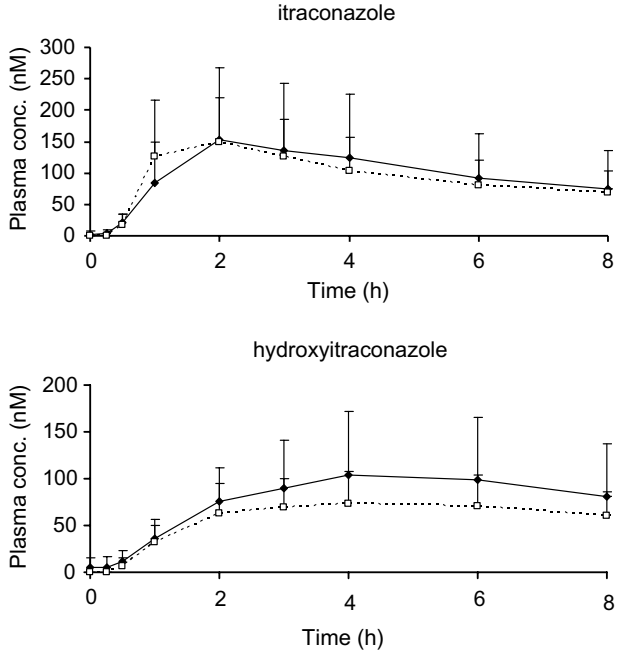

Fig. 6. Plasma concentration–time profiles of itraconazole and hydroxyitraconazole after single dosing with OMS loaded with itraconazole (◆), Sporanox® (□) in dogs ($n = 5$). Concentrations were dose normalized to the amount provided by the OMS capsules (20.4 ± 0.1 mg) (average ± SD).

4. Conclusions

In the present study, the biopharmaceutical performance of ordered mesoporous silica material (OMS) as a carrier for the poorly water soluble drug itraconazole was investigated. When itraconazole was loaded into OMS, the adsorption led to a molecular dispersion of the drug. Release of itraconazole from OMS in aqueous environment occurred faster than the dissolution of the pure drug, circumventing its slow dissolution kinetics. When non-sink conditions were established, OMS was able to establish a supersaturated solution. For the first time, the performance of such inorganic carriers was examined *in vivo*. In rabbits as

Table 1
Summary of the AUC (nM h) values for itraconazole and hydroxyitraconazole obtained with rabbits and dogs, after oral dosing with three different formulations ($n = 5$, average + SD)

	Rabbits $AUC_{0-24\ h}$ (nM h)		Dogs $AUC_{0-8\ h}$ (nM h)	
	Itraconazole	Hydroxyitraconazole	Itraconazole	Hydroxyitraconazole
OMS	1069 ± 278	1179 ± 388	681 ± 566	533 ± 329
Sporanox®	1155 ± 424	1329 ± 544	760 ± 364	470 ± 218
Crystalline itraconazole	521 ± 159	572 ± 177	0 ± 0	0 ± 0

Values were normalized for the dose used with OMS (8.1 ± 0.1 mg for rabbits, 20.4 ± 0.1 mg for dogs).

well as in dogs, the systemic availability of the drug was boosted significantly. Furthermore, all results obtained with OMS were comparable with the marketed product Sporanox®. These results evidence that ordered mesoporous silica is a promising carrier to achieve enhanced oral bioavailability for drugs with extremely low water solubility.

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