Oral Bioavailability in Pigs of a Miconazole/Hydroxypropyl- γ -cyclodextrin/L-tartaric Acid Inclusion Complex Produced by Supercritical Carbon Dioxide Processing

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ABSTRACT

The objective of this study was to determine the pharmacokinetic parameters of miconazole after oral administration of a miconazole/hydroxypropyl- γ -cyclodextrin(HP γ CD)/L-tartaric acid inclusion complex produced by supercritical carbon dioxide processing. The pharmacokinetics of the miconazole ternary complex (CPLX), of the corresponding physical mixture (PHYS), and of miconazole alone (MICO) were compared after oral administration. Six mixed-breed pigs received each formulation as a single dose (10 mg miconazole/kg) in a crossover design. Miconazole plasma concentrations were determined by a high-performance liquid chromatography method. Preliminary in vitro dissolution data showed that CPLX exhibits a faster and higher dissolution rate than either PHYS or MICO. Following CPLX oral administration, mean area under the plasma concentration curve ($AUC_{0-\infty}$) for miconazole was $95.0 \pm 55.8 \mu\text{g}/\text{min}/\text{mL}$, with the peak plasma concentration (C_{max} $0.59 \pm 0.39 \mu\text{g}/\text{mL}$) at 19.30 minutes. The $AUC_{0-\infty}$ and C_{max} values were significantly higher than those after oral administration of PHYS ($AUC_{0-\infty}$ $38.5 \pm 12.7 \mu\text{g}/\text{min}/\text{mL}$ and C_{max} $0.24 \pm 0.08 \mu\text{g}/\text{mL}$; $P < .1$) and of MICO ($AUC_{0-\infty}$ $24.1 \pm 14.0 \mu\text{g}/\text{min}/\text{mL}$ and C_{max} $0.1 \pm 0.05 \mu\text{g}/\text{mL}$; $P < .1$). There were also significant differences between PHYS and MICO ($P < .1$). The results of the study indicate that CPLX shows improved dissolution properties and a higher relative oral bioavailability compared with PHYS and MICO.

KEYWORDS: oral bioavailability, supercritical carbon dioxide, multicomponent inclusion complex, cyclodextrin, miconazole, dissolution test

INTRODUCTION

Miconazole is a well-known broad-spectrum antimycotic drug. Because its poor water solubility and poor dissolution

properties prevent its gastrointestinal absorption, the clinical indications of miconazole are limited to topical mycosis. Miconazole can be used intravenously to treat systemic mycosis. The preparation of miconazole IV solutions required the use of polyoxyl-35 castor oil (Cremophor EL) to achieve a micellar solution and to enhance miconazole solubility; however, this excipient is responsible for anaphylactoid hypersensitivity reactions.¹ Consequently, several authors successfully used cyclodextrins (CDs) as the solubilizing agent² to improve miconazole solubility by means of inclusion complexes.³⁻⁵ Using CDs enabled the development of a miconazole solution with the same miconazole concentration as that of the micellar solution.⁶

In oral drug delivery, CDs and their derivatives can modify the absorption of the drug by the gastrointestinal tract, thus increasing the bioavailability of poorly water-soluble active compounds.⁷ To date, few attempts have been made to improve the oral bioavailability of miconazole and results are inconsistent. Tenjarla et al observed an increase of miconazole bioavailability when a solid inclusion complex was administered orally to mice,⁸ but Hostetler et al observed that a CD solution of miconazole did not improve its bioavailability after human administration.⁹ Supercritical carbon dioxide (SCCO₂) has been successfully used to produce genuine CD inclusion complexes in recent years.¹⁰⁻¹² This particular processing allows the formation of solid inclusion compounds without the use of water or organic solvents. After such processing, improved dissolution profiles have been achieved by formation of drug/CD inclusion compounds.

In a previous study,¹³ we demonstrated that the formation of a stable miconazole/CD inclusion complex is possible using SCCO₂ and that an acidic ternary compound, such as an organic acid, can modify the miconazole inclusion yield. The complexation of miconazole into CD improved its dissolution profile, and the presence of a genuine inclusion complex was observed by infrared spectroscopy. The present study was designed to evaluate the influence of both hydroxypropyl- γ -cyclodextrin (HP γ CD) and L-tartaric acid (which dramatically promote the miconazole inclusion during the supercritical process) on miconazole bioavailability in pigs and the need of a prior miconazole complexation rather than a simple physical mixture. All the

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formulations were compared with a miconazole capsule. The pig was used because this nonprimate animal model resembles the human better than any other nonprimate animal species with regard to eating behavior, anatomy, and physiology of the gastrointestinal tract.¹⁴ The pig can therefore be considered as a suitable model for performance evaluation of oral pharmaceutical products.¹⁵ Different authors currently have used the pig to evaluate a range of pharmaceutical dosage forms in bioavailability studies.^{16,17}

MATERIALS AND METHODS

Animals

Six mixed-breed 3-month-old pigs (3 males and 3 females), weighing 31 to 45 kg at the start of the study, were used. Pigs were housed indoors and fed a drug-free commercial pellet diet. Pigs had free access to drinking water. All pigs were in good health as determined by physical examination before drug administration. Animal housing, handling, and experimental design were approved by the Ethics Committee of the University of Mons-Hainaut (Mons, Belgium).

Drug and Products

Miconazole base (*Eur Ph* 4th ed) was from Janssen Pharmaceutica (Beerse, Belgium). HP γ CD (Cavasol W8 HP Pharma, Degree of Substitution (DS) 0.62, batch no. 83P002) was supplied by Wacker GmbH (Munich, Germany). Lactose monohydrate (*Eur Ph* 4th ed) was from Certa (Braine-l'Alleud, Belgium). L-tartaric acid (*Eur Ph* 4th ed) was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

Dosage Form Preparations

Reference Dosage Form (Miconazole Alone; MICO)

A mixture of miconazole and lactose monohydrate was prepared by mixing exact amounts of miconazole and lactose in a mortar in order to achieve a miconazole content equal to 20% (wt/wt). Then, no. 0 hard-gelatin capsules were filled with this mixture in order to achieve a quantity corresponding to 50 mg miconazole.

Physical Mixture Form (PHYS)

The physical mixture of miconazole base, HP γ CD, and L-tartaric acid was prepared by mixing calculated and exactly weighed amounts of miconazole, CD, and acid in equimolar ratio in a mortar.

PHYS was introduced into the no. 0 hard-gelatin capsules in order to achieve a quantity corresponding to 50 mg miconazole base.

Solid Inclusion Complex Form (CPLX)

The miconazole base/HP γ CD/L-tartaric acid inclusion complex was prepared using supercritical carbon dioxide processing in a SUPREX SF Extractor Autoprep 44 (Suprex, Pittsburgh, PA) described elsewhere.¹⁰ A 1-mL cartridge was filled with 400 mg of physical mixture of miconazole/HP γ CD/L-tartaric acid 1:1:1 (mol:mol:mol). The content was pressurized in ± 1 minute and left in a static mode at 30 MPa and 125°C for 60 minutes. At the end of the experiment, the cartridge was depressurized within 15 seconds. The cartridge content was emptied, ground, and homogenized in a mortar.¹³

CPLX was introduced into the no. 0 hard-gelatin capsules in a quantity equivalent to 50 mg miconazole.

Experimental Design and Drug Administration

The oral administration study was performed in crossover, following the experimental design presented in Table 1. All administrations were followed by a 14-day washout period. The pigs were weighed on the day of drug administration in order to adapt the miconazole dose. Each animal, was deprived of food 12 hours before the experiment, received a dose equal to 10 mg miconazole/kg body weight for the 3 formulations by means of a balling gun. Blood samples were collected from the jugular vein by venipuncture into BD vacutainer tubes containing heparin (Alost, Belgium) before dosing and at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 480, and 600 minutes after oral administration.

All blood samples were centrifuged for 15 minutes at 3000 rpm at 4°C, and the plasma was kept at -20°C until high-performance liquid chromatography (HPLC) assay.

In Vitro Dissolution Testing

The in vitro dissolution profiles of MICO, PHYS, and CPLX were performed according to the paddle method described in the *European Pharmacopoeia*, 4th ed, using a dissolution test apparatus, Sotax AT7 (Basel, Switzerland), connected with a Watson-Marlow 505Du peristaltic pump (Falmouth, UK) to a Hitachi 3000 flow-through UV spectrophotometer (Tokyo, Japan).

A quantity equivalent to 13 mg of miconazole was exactly weighed, introduced into a hard-gelatin capsule, and then immersed by means of a sinker into 900 mL of dissolution medium containing a 50-mM pH 6.8 phosphate buffer. This medium was stirred at 100 rpm and set at $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$. Every 2 minutes, the medium was pumped at a rate of 15 mL/min and passed through a 0.47- μm filter; then the absorbance of this solution was measured at 230 nm. Each experiment was performed in duplicate.

Table 1. Experimental Design for the Oral Administration of the Solid Dosage Forms Containing Miconazole*

Pig	First Oral Administration	Second Oral Administration	Third Oral Administration
p1	MICO	CPLX	PHYS
p2	MICO	PHYS	CPLX
p3	PHYS	CPLX	MICO
p4	PHYS	MICO	CPLX
p5	CPLX	PHYS	MICO
p6	CPLX	MICO	PHYS

*MICO indicates miconazole base; PHYS, miconazole base/HP γ CD/L-tartaric acid physical mixture; and CPLX, miconazole base/HP γ CD/L-tartaric acid complex.

The standard solution was prepared by dissolving an exactly weighed quantity of miconazole in a mixture of methanol and pH 6.8 50-mM phosphate buffer (50/50 vol: vol) to achieve the final concentration of 25 μ g/mL.

The pH of the medium was checked at the end of the experiment.

Bioanalysis Method and Validation

Miconazole was assayed using an HPLC method derived from Piel et al.¹⁸ A partial validation has been realized following the Food and Drug Administration recommendations.¹⁹

The HPLC system (Merck-Hitachi, Tokyo, Japan) consisted of an L-6000 pressure pump, an L-7200 autosampler, an L-7350 column oven, an L-7400 UV detector, and a D-7000 interface. The system was controlled by a computer running the HPLC System Manager Version 4.0 acquisition software developed by Merck-Hitachi.

Chromatography was performed using an octylsilane (C₈) phase Lichrospher 60 RP-Select B (12.5 cm $\times$ 4 mm inner

diameter, 5 μ m) column (Merck, Darmstadt, Germany) protected by a guard-column packed with the same stationary phase as the analytical column, all maintained at 30°C. The mobile phase consisted of a degassed mixture of acetonitrile and 50-mM pH 7.2 sodium acetate buffer 70/30 (vol/vol); its flow rate was adjusted at 1.0 mL/min. The eluent was monitored spectrophotometrically at 230 nm.

Plasma samples of 250 μ L were mixed with 250 μ L acetonitrile to precipitate the plasmatic proteins, then vortexed for 30 seconds and kept standing for 5 minutes at room temperature. The suspension was then centrifuged for 15 minutes at 4000 rpm and 50 μ L of supernatant was chromatographed.

Standard curves were constructed in the concentration range of 0.05 to 20 μ g of miconazole/mL in drug-free plasma. The partial validation involves the determinations of recovery and of intra-assay precision at 3 concentrations: 0.15, 5, and 15 μ g/mL. Blank pig plasma and pig plasma spiked with 5 μ g/mL of miconazole were assayed (Figure 1). The retention time of miconazole was approximately 4.5 minutes. No interference peaks of endogenous constituents from plasma were observed at the miconazole retention time. The validation data, reported in Table 2, show that this method, after log-log transformation, was linear, precise, accurate, and reproducible. The correlation coefficient (r^2) was 0.9996, the intercept was 5.038 ± 0.005 , and the slope was 0.988 ± 0.005 . The limits of detection (LOD) and of quantification (LOQ) were 0.013 μ g/mL and 0.044 μ g/mL, respectively.

Pharmacokinetics and Statistical Analysis

The relevant pharmacokinetic parameters such as area under the curve during the sampling period (AUC₀₋₆₀₀), the AUC extrapolated to the infinite (AUC_{0- ∞}), the maximum plasma concentration (C_{max}), the time to reach peak con-

Figure 1. Chromatograms obtained after injection of blank pig plasma (A) and of pig plasma spiked with 5 μ g/mL miconazole (B).

Table 2. Validation of Miconazole Assay*

Parameter	Value
Quantification range ($\mu\text{g/mL}$)	0.050-20
Linearity (r^2)	0.9996
Slope	0.988 ± 0.005
Intercept	5.038 ± 0.005
Recovery (Mean $\pm$ RSD, %)	
0,150 $\mu\text{g/mL}$	97.72 ± 5.08
5 $\mu\text{g/mL}$	98.00 ± 2.78
15 $\mu\text{g/mL}$	98.48 ± 2.46
Intra-assay precision (% RSD)	
0,150 $\mu\text{g/mL}$	2.78
5 $\mu\text{g/mL}$	1.46
15 $\mu\text{g/mL}$	0.96
LOQ ($\mu\text{g/mL}$)	0.044
LOD ($\mu\text{g/mL}$)	0.013

*RSD indicates relative standard deviation; LOQ, limits of quantification; and LOD, limits of detection.

centration ($T_{\max}$), and the relative bioavailability (F_{rel}) were determined for each animal. Pigs were excluded from the pharmacokinetic analysis when the individual AUC value was higher or lower than 1 SD ($n = 6$ pigs) in the tested formulations. Based on this, 1 pig was excluded after the oral administration of PHYS and CPLX.

The pharmacokinetic parameters are reported as mean $\pm$ SD. For each calculated parameter, mean values were compared after log-transformation in order to normalize the distribution. A 2-way analysis of variance (ANOVA) followed by Scheffe's test was performed. A P value $\leq .1$ was considered to be statistically different.

RESULTS AND DISCUSSION

Determination of the Inclusion Yield in the Miconazole Complex

The miconazole inclusion yield was determined in the complex using the differential solubility method, which

Table 3. Miconazole Inclusion Yield Determined by Differential Solubility Method in the Complex Produced by Supercritical Carbon Dioxide Processing*

Total Miconazole Content (% wt/wt)	Free Miconazole Content (% wt/wt)	Inclusion Yield (%)
20.6 ± 0.4	1.6 ± 0.1	92.2 ± 0.4

*Values are mean $\pm$ SD, $n = 3$.

has been described by Van Hees et al.²⁰ This method was successfully applied to the miconazole complexes and was validated in a previous study.¹³ The results are shown in Table 3. The total miconazole content was equal to 20.6% and its free content to 1.6%. Since the miconazole inclusion was 92.2%, the miconazole inclusion was nearly complete in the complex produced by supercritical processing.

In Vitro Dissolution Testing

Figure 2 shows the in vitro release profiles of miconazole from the different formulations: MICO, PHYS, and CPLX. Before comparing the dissolution curves, it is interesting to note that the pH value of the dissolution medium remained unchanged after the different runs. The dissolution profile of the CPLX exhibited a higher $C_{\max}$ ($12.2 \pm 0.7 \mu\text{g/mL}$) than that of the other compounds ($9.7 \pm 0.7 \mu\text{g/mL}$ for PHYS and $5.8 \pm 0.3 \mu\text{g/mL}$ for MICO). The values of the AUC_{0-60} , determined by trapezoidal rule during the sampling period (60 minutes), follow the same trend: AUC_{0-60} of the CPLX is equal to $580.6 \pm 38.0 \mu\text{g/min/mL}$ versus $474.1 \pm 39.2 \mu\text{g/min/mL}$ for PHYS and $283.5 \pm 30.7 \mu\text{g/min/mL}$ for MICO. The times required to reach maximum concentration ($T_{\max}$) move in the opposite direction with the following values: 11.0 ± 7.1 minutes for the CPLX, 17.0 ± 4.3 minutes for the PHYS, and 49.0 ± 4.2 minutes for MICO. The presence of CD and of the acid in the physical mixture improves the dissolution properties of miconazole by the in situ formation of a soluble miconazole/CD inclusion complex and of a favorable acidic micro-pH resulting from the dissolution of L-tartaric acid. So, the $C_{\max}$ of PHYS is twice higher than that of MICO, and this peak concentration appears earlier. Moreover, the same observations can be made for the CPLX. Indeed, the $C_{\max}$ and the AUC_{0-60} of CPLX are the highest, but the

Figure 2. In vitro dissolution profiles of miconazole for the 3 formulations: miconazole base ($\blacktriangledown$), miconazole base/HP γ CD/L-tartaric physical mixture ($\blacksquare$), and miconazole base/HP γ CD/L-tartaric complex ($\bullet$).

Figure 3. Mean plasma concentration versus time after oral administration in pigs of a single dose of 10 mg/kg of miconazole base (▼, $n = 6$), miconazole base/HP γ CD/L-tartaric physical mixture (■, $n = 5$), and miconazole base/HP γ CD/L-tartaric complex (▲, $n = 5$).

$T_{\max}$ is the lowest. In this case, miconazole is already included into the CD cavity and the inclusion complex improves the dissolution of miconazole. At the end of the dissolution test, for both PHYS and CPLX, a decrease in the miconazole concentration is observed. This decrease is the result of the dilution of the miconazole/CD complex, of the CD, and of the acid, which promotes the dissociation of the complex according to its binding constant; finally, miconazole precipitates in order to reach its solubility. These results show that the presence of CD and of L-tartaric acid in the physical mixture and the inclusion of the active compound into the CD cavity can enhance the dissolution rate.

Pharmacokinetics of Miconazole After Oral Administration: Comparison of the 3 Formulations

In order to evaluate the influence of the inclusion complex (CPLX) and of the physical mixture (PHYS) with HP γ CD and L-tartaric acid as acidic ternary compound, an in vivo study was conducted in pigs. Miconazole base was used as reference (MICO).

The miconazole mean plasma concentrations over time after oral administration are shown in Figure 3. The mean miconazole pharmacokinetic parameters are summarized in Table 4. The plasma levels of miconazole after administration of the CPLX are clearly higher than those achieved with an equal dose of PHYS and of MICO. In particular, mean miconazole plasma peak concentrations of 0.59, 0.24, and 0.10 $\mu\text{g/mL}$ were observed for CPLX, PHYS, and MICO respectively. So, $C_{\max}$ for CPLX is about 6 times higher than that of MICO and 2 times higher than that of PHYS ($P < .1$). The times required to reach peak

concentration do not agree with those observed during the in vitro dissolution test. Indeed, $T_{\max}$ for CPLX is 19.3 ± 12.8 minutes; for PHYS, 12.7 ± 4.1 minutes; and for MICO, 41.8 ± 38.4 minutes. There are also statistical differences between the 3 compounds ($P < .1$). The AUC values follow the same trend as the $C_{\max}$ values do. Indeed, the AUC_{0-600} of CPLX is 4.5-fold higher than MICO and 3-fold higher than PHYS. Similarly, $\text{AUC}_{0-\infty}$ for CPLX is 3.9 times higher than for MICO and 2.5 times higher than for PHYS. There are statistical differences between these area values for all the tested products ($P < .1$). However,

Table 4. Pharmacokinetic Parameters (mean $\pm$ SD) Obtained From Plasma Concentrations of Miconazole After Oral Administration of Miconazole Base (MICO), Miconazole Base/HP γ CD/L-Tartaric Physical Mixture (PHYS), and Miconazole Base/HP γ CD/L-Tartaric Complex (CPLX) to pigs*

	MICO	PHYS	CPLX
$C_{\max}$ ($\mu\text{g/mL}$)	0.10 ± 0.05	$0.24 \pm 0.08^{\dagger}$	$0.59 \pm 0.39^{\ddagger\dagger}$
$T_{\max}$ (minutes)	41.8 ± 38.4	12.7 ± 4.1	$19.3 \pm 12.8^{\ddagger\dagger}$
AUC_{0-600} ($\mu\text{g/min/mL}$)	20.4 ± 12.7	33.7 ± 14.9	$90.4 \pm 53.1^{\ddagger\dagger}$
$\text{AUC}_{0-\infty}$ ($\mu\text{g/min/mL}$)	24.1 ± 14.0	$38.5 \pm 12.7^{\dagger}$	$95.0 \pm 55.8^{\ddagger\dagger}$
F_{rel} (%)	—	160	394

* $C_{\max}$ indicates maximum plasma concentration; $T_{\max}$, time to reach peak concentration; AUC_{0-600} , area under the curve during the sampling period; $\text{AUC}_{0-\infty}$, AUC extrapolated to the infinite; and F_{rel} , relative bioavailability; $n = 6$ except for the physical mixture and the complex where $n = 5$.

† Significantly different from MICO ($P < .1$).

‡ Significantly different from PHYS ($P < .1$).

the improvement in miconazole bioavailability depends on the presence of CD, either in the inclusion complex or in the physical mixture.

CDs have been used for many years to increase oral bioavailability by increasing the dissolution rate of the native drug.²¹ When a formulation containing a solid CD inclusion complex is administered, the complex dissolves and equilibrium between the dissolved complex and the free drug is achieved in function of the binding constant. The dissociation of the inclusion complex is an important factor, since only the free drug is able to penetrate into the lipophilic membranes of the gastrointestinal tract, and to finally reach the systemic circulation. So, the release of the drug depends on the dissociation constant of the complex and increases in the presence of endogenous compounds that can act as competing agents for the CD cavity and thus shift the drug.²² The increased miconazole dissolution would lead to efficient passive absorption by producing high concentrations of free drug at the surface of the membrane of the gastrointestinal tract. The miconazole inclusion complex produces higher free miconazole concentrations than the concentrations derived from either the physical mixture or from miconazole alone. The pharmacokinetic parameters for miconazole show that the complex provides more extensive absorption in terms of C_{max} , AUC, and absolute bioavailability. Compared with miconazole, the physical mixture also increases miconazole bioavailability. The mechanism leading to this modification resembles that of the complex. Indeed, after oral administration of the physical mixture, there is in situ formation of an inclusion complex, which promotes the miconazole dissolution rate leading to an enhancement of bioavailability.^{23,24} But this formation is not complete and, moreover, endogenous competing agents can prevent this complex formation. This is probably why the miconazole relative bioavailability of the physical mixture is lower than that of the inclusion complex.

CONCLUSION

In the first part of this study, dissolution tests were performed, and the results show that a miconazole inclusion complex produced by means of $SCCO_2$ gives a faster dissolution rate and a higher AUC value than the corresponding physical mixture and miconazole.

Oral administration reveals that miconazole exhibits poor pharmacokinetic and bioavailability performances. Administrations of the physical mixture, and particularly of the inclusion complex, were characterized by a better absorption efficacy than that of miconazole alone. Taken together, the results suggest that the inclusion into CDs may be important for the modulation of miconazole release and its dissolution from an oral dosage form.

Last, this study clearly shows that the multicomponent complex between miconazole, HP γ CD, and L-tartaric acid produced by means of $SCCO_2$ is able to increase the drug's oral bioavailability.

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