In Vitro Investigation of the Hepatobiliary Disposition Mechanisms of the Antifungal Agent Micafungin in Humans and Rats

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ABSTRACT:

The purpose of the present study was to elucidate the transport mechanisms responsible for elimination of micafungin, a new semisynthetic echinocandin antifungal agent, which is predominantly cleared by biliary excretion in humans and rats. In vitro studies using sandwich-cultured rat and human hepatocytes were conducted. Micafungin uptake occurred primarily (~75%) by transporter-mediated mechanisms in rat and human. Micafungin uptake into hepatocytes was inhibited by taurocholate ($K_i = 61 \mu\text{M}$), Na^+ depletion (45–55% reduced), and $10 \mu\text{M}$ rifampin (20–25% reduced); these observations support the involvement of Na^+ -taurocholate-cotransporting polypeptide (NTCP/Ntcp) and, to a lesser extent, organic anion-transporting polypeptides in the hepatic uptake of micafungin. The in vitro biliary clearance of micafungin, as measured by the B-CLEAR technique, amounted to 14 and $19 \mu\text{l}/(\text{min} \cdot \text{mg protein})$ in human and rat, respectively. In vitro biliary excretion of micafungin

was reduced by 80 and 75% in the presence of the bile salt export pump (BSEP) inhibitors taurocholate ($100 \mu\text{M}$) and nefazodone ($25 \mu\text{M}$), respectively. Biliary excretion of micafungin also was reduced in the presence of breast cancer resistance protein inhibitors [*N*-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918) ($10 \mu\text{M}$) and fumitremorgin C ($10 \mu\text{M}$)]. In vitro biliary excretion of micafungin was not significantly altered by coinubation with P-glycoprotein or multidrug resistance-associated protein 2 inhibitors. These results suggest that NTCP/Ntcp and BSEP/Bsep are primarily responsible for hepatobiliary disposition of micafungin in human and rat. Interference with hepatic bile acid disposition could be one mechanism underlying hepatotoxicity associated with micafungin in some patients.

Introduction

Micafungin is a new semisynthetic echinocandin-type antifungal drug with activity against both *Candida* and *Aspergillus* species that relies on inhibition of β -1,3-glucan synthesis to exert its therapeutic

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effect. Because β -1,3-glucan is a unique but essential component of the fungal cell wall, micafungin has a superior activity/toxicity profile compared with the polyene antifungal drug amphotericin B, with less drug interactions than the azole antimycotic drugs. Pharmacokinetic studies with [^{14}C]micafungin demonstrated that approximately 90% of the plasma clearance occurs via biliary elimination of the parent and minor metabolites (Hebert et al., 2005a), whereas urinary excretion is a minor elimination route in humans and rats (Kaneko et al., 2002; Yamato et al., 2002; Carver, 2004). Like caspofungin and anidulafungin, micafungin is a peptide-like compound. Because of the presence of a sulfate group, micafungin is negatively charged at physiological pH (Fig. 1). Micafungin displays high plasma protein binding (>99%) in humans and animals (Carver, 2004).

Mechanisms underlying the hepatobiliary disposition of micafungin in humans remain to be elucidated. Drug-drug interaction studies in humans and in rats have been useful but not sufficient to support our understanding of its disposition in human populations. Sakaeda et al.

ABBREVIATIONS: P-gp, P-glycoprotein; AUC, area under the plasma concentration versus time curve; MRP/Mrp, multidrug resistance-associated protein; OATP/Oatp, organic anion-transporting polypeptide; NTCP/Ntcp, Na^+ -taurocholate-cotransporting peptide; BCRP/Bcrp, breast cancer resistance protein; BSEP/Bsep, bile salt export pump; MK571, 3-[[3-[2-(7-chloroquinolin-2-yl)vinyl]phenyl]-(2-dimethylcarbamoyl-ethylsulfanyl)methylsulfanyl] propionic acid; GF120918, *N*-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide; CDFDA, 5-(and 6)-carboxy-2',7'-dichlorofluorescein diacetate; SCRH, sandwich-cultured rat hepatocytes; SCHH, sandwich-cultured human hepatocytes; HPLC, high-performance liquid chromatography; BEI, biliary excretion index.

FIG. 1. Chemical structure of micafungin.

(2005) reported that micafungin is not a substrate or inhibitor for human P-glycoprotein (P-gp) in a multidrug resistance 1-overexpressing cell line. Clinical studies by Hebert et al. (2005a,b) showed that cyclosporine A significantly increased micafungin exposure (AUC), whereas tacrolimus had no effect. In rats, intravenous administration of cyclosporine A reduced the systemic clearance, volume of distribution at steady state, and the biliary clearance of micafungin. In a recent study, Abe et al. (2008b) reported a role for multidrug resistance-associated protein 2 (Mrp2, *Abcc2*) in the biliary excretion of micafungin in the rat. These authors demonstrated that micafungin biliary clearance was significantly decreased (by ~60%) in Eisai hyperbilirubinemic Mrp2-deficient rats [TR(–/–)] compared with wild-type animals.

On the basis of clinical and preclinical data, the chemical structure, and physicochemical properties of micafungin, it is our hypothesis that micafungin hepatobiliary clearance is mediated by hepatic drug uptake transporters such as those belonging to the organic anion-transporting polypeptide (OATP, *SLCO*) family and/or the Na⁺-taurocholate-cotransporting polypeptide (NTCP/Ntcp, *SLC10A1*) and by one or more of the following efflux transporters on the canalicular membrane: 1) MRP2/Mrp2 (*ABCC2/Abcc2*); 2) breast cancer resistance protein (BCRP/Bcrp, *ABCG2/Abcg2*); and 3) bile salt export pump (BSEP/Bsep, *ABCB11/Abcb11*). The in vitro studies performed with sandwich-cultured hepatocytes in the present study demonstrate that NTCP and BSEP play a major role in the hepatobiliary disposition of micafungin.

Materials and Methods

Chemicals and Reagents. Micafungin sodium was a gift from Dr. John Perfect at Duke University. [³H]Taurocholic acid (5 Ci/mmol) was obtained from PerkinElmer Life and Analytical Sciences (Waltham, MA). Choline chloride, collagenase (type IV), dexamethasone, fumitremorgin C, 3-[[3-[2-(7-chloroquinolin-2-yl)vinyl]phenyl]-(2-dimethylcarbamoyl)ethylsulfanyl]methylsulfanyl] propionic acid (MK571), nefazodone, rifampin, and taurocholic acid were purchased from Sigma-Aldrich (St. Louis, MO). *N*-(4-[2-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918, Elacridar) was a gift from GlaxoSmithKline (Uxbridge, Middlesex, UK), whereas 5-(and 6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) was purchased from Invitrogen (Carlsbad, CA). ITS+ (insulin, transferrin, selenious acid, and linoleic acid bound to bovine serum albumin) culture supplement and BioCoat plates were purchased from BD Biosciences Discovery Labware (Bedford, MA). Williams' E medium or Dulbecco's modified Eagle's medium, fetal bovine serum, penicillin G sodium, and streptomycin sulfate, modified essential medium nonessential amino acids, and *L*-glutamine were obtained from Invitrogen. Standard buffer (Plus buffer) consisted of Hanks' balanced salt solution containing 10 mM Hepes and was adjusted to pH 7.4. Ca²⁺-free buffer (Minus buffer) consisted of Ca²⁺/Mg²⁺-free Hanks' balanced salt solution containing 1 mM EGTA and 10 mM Hepes and was adjusted to pH 7.4. Na⁺-free

buffer consisted of 140 mM choline chloride, 10 mM Hepes, 1 mM CaCl₂, 1 mM MgSO₄, 10 mM Tris, 5 mM KCl, and 5 mM glucose.

Animals. Male Wistar rats (240–300 g) were used for hepatocyte preparations. Animals had free access to water and food before surgery. All animal procedures complied with the guidelines of the Institutional Animal Care and Use Committee (Katholieke Universiteit Leuven and University of North Carolina at Chapel Hill). TR(–/–) Wistar rats (206–346 g) originating from the breeding stock obtained from Dr. Mary Vore (University of Kentucky, Lexington, KY) were bred at the University of North Carolina (Chapel Hill, NC) and were used for hepatocyte preparations by the Cellular and Metabolism and Transport Core Facility at the University of North Carolina Eshelman School of Pharmacy.

Preparation of Sandwich-Cultured Human and Rat Hepatocytes.

Freshly isolated human hepatocytes from three liver donors were used. The following donor data were available: donor 1, 16-year-old white male; donor 2, 42-year-old white female; and donor 3, 36-year-old white male. Hepatocytes were isolated by CellzDirect (Durham, NC), plated in 24-well BioCoat culture plates overlaid with Matrigel, and cultured at 37°C in a humidified incubator with 95% O₂-5% CO₂ in serum-free Williams' E medium supplemented with gentamicin, dexamethasone, ITS+ culture supplement, *L*-glutamine, and Hepes. Medium was changed every day until the transport studies were conducted. For rat hepatocytes, cell isolation was achieved by using a two-step collagenase perfusion as described previously (Abe et al., 2008c; Ye et al., 2008), and viability was >90% with a yield of 4 to 6 × 10⁸ cells/liver. After isolation, hepatocytes were seeded in 12-well plates at 1 million cells/well and cultured as described previously by Ye et al. (2008).

Uptake Study. Day 1 sandwich-cultured rat hepatocytes (SCRH) or day 6 sandwich-cultured human hepatocytes (SCHH) were used to measure micafungin uptake. Hepatocytes (three wells per condition) were preincubated with 1 ml/well (12-well plate) or 0.5 ml/well (24-well plate) of standard buffer for 10 min at 37°C, followed by another 10-min preincubation in the absence (control) or in the presence of rifampin (10 μM), taurocholate (100 μM), or Na⁺-free/choline buffer (prepared as described by Su et al., 2004). In parallel, cells were incubated with standard buffer at 4°C. Subsequently, micafungin (10 μM) was added to all inhibitor-treated cells or untreated cells (control) to initiate uptake for 10 min (previously determined to be linear). After the incubation, cells were rinsed three times with 1 ml/well of ice-cold standard buffer. After rinsing, micafungin was extracted from hepatocytes by shaking the plates for 20 min at room temperature after addition of 1 ml of 10% formic acid in acetonitrile (v/v) containing 1 μM diclofenac as analytical internal standard. The extracted micafungin samples were centrifuged for 10 min at 10,000 rpm (14,000g), and supernatant was dried under air before reconstitution in 0.5 ml of 10% acetonitrile-containing 5% formic acid (v/v). Aliquots (100 μl) were analyzed for quantitative determination of micafungin using high-performance liquid chromatography (HPLC) coupled with fluorescence and ultraviolet detection. Uptake was normalized to the protein content of the hepatocytes, determined in at least three separate wells by lysing the hepatocytes in these wells with 0.5 ml/well of 0.5% Triton X-100 solution and analyzing the cell lysate by the bicinchoninic acid method using bovine serum albumin as standard (0.2–1.0 mg/ml). All uptake data were corrected for nonspecific binding to collagen-coated, hepatocyte-free culture wells.

Concentration-dependent inhibition of [³H]taurocholate (2.5 μM) or micafungin (10 μM) uptake by micafungin or taurocholate, respectively, was measured by treating the sandwich-cultured rat hepatocytes with taurocholate (0–200 μM) or micafungin (0–500 μM) in the presence of the other. After a 10-min incubation with the putative inhibitor, accumulated micafungin was extracted and quantitatively determined as described above, and accumulated [³H]taurocholate was determined after lysing hepatocytes with 0.5 ml of 0.5% Triton X-100 solution and analyzing cell lysates by liquid scintillation spectrometry (LS50B; PerkinElmer Life and Analytical Sciences). Corresponding IC₅₀ values were estimated according to eq. 1 from the best fit to substrate uptake data as a percentage of no inhibitor control versus inhibitor concentrations by using WinNonlin software.

The kinetics of micafungin uptake in day 1 SCRH or day 6 SCHH were measured by incubating hepatocytes with 1 to 500 μM micafungin for 10 min, and processing samples as described above. The best fits to micafungin uptake data were obtained by nonlinear regression analysis using WinNonlin to determine the kinetic parameters for the saturable (Michaelis-Menten: *K_m* and *V_{max}*) and nonsaturable (*Cl_d*) uptake according to eq. 2 (see *Data Analysis*).

Micafungin uptake at 4°C or at 37°C in the presence of choline buffer and 10 μ M rifampin was measured at various micafungin concentrations and subtracted from total uptake measured at 37°C under control conditions to determine the net uptake. The net uptake kinetic parameters were determined by fitting the Michaelis-Menten model in eq. 3 to the data.

Biliary Excretion by Measuring Accumulation (B-CLEAR). SCRH (day 4) or SCHH (day 8) were rinsed with 1.0 ml (12-well plate) or 0.5 ml (24-well plate) of standard buffer (containing $\text{Ca}^{2+}/\text{Mg}^{2+}$; Plus buffer) or $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free buffer (containing 1 mM EGTA; Minus buffer) and preincubated with the same buffers for 10 min. All wells were then incubated with 10 μ M micafungin in Plus buffer for 10 min. For conditions with modulators (inhibitors), hepatocytes were treated during the preincubation phase as well as during the incubation phase with taurocholate (50 and 100 μ M), nefazodone (25 μ M), GF120918 (1 and 10 μ M), fumitremorgin C (10 μ M), CDFDA (100 μ M), or MK571 (25 μ M). After the incubation, hepatocytes were rinsed rapidly three times with 1 ml of ice-cold Plus buffer. After rinsing, micafungin was extracted from the cells + bile (for hepatocytes incubated in Plus buffer) and cells (for hepatocytes incubated in Minus buffer), and analyzed as described above.

Biliary Excretion by Measuring Efflux after Preloading Hepatocytes. SCRH (day 4) or SCHH (day 8) were preincubated for 10 min with Plus buffer and then incubated with 10 μ M micafungin in Plus buffer to load hepatocytes with micafungin (loading phase). Then hepatocytes were rapidly rinsed with ice-cold Plus buffer, followed by either Plus buffer or Minus buffer at 37°C to initiate micafungin efflux to the extracellular medium. Incubation with Plus buffer allows measurement of efflux from the cells across the sinusoidal membrane only, whereas incubation with Minus buffer allows measurement of total efflux from the cells across both sinusoidal and canalicular membrane domains. When the effect of (canalicular) transporter inhibitors on micafungin efflux was evaluated, they were added during both the loading and efflux phase to ensure maximal exposure of canalicular transporters to these inhibitors. At the end of the efflux phase, extracellular media were collected and centrifuged for 3 min at 10,000 rpm, and micafungin concentrations were determined as described below. All results were corrected for incubation time (efflux phase) and protein content.

Determination of Micafungin by HPLC Coupled with Fluorescence and UV Detection. Micafungin and diclofenac (internal standard) were separated by HPLC using a C8 XTerra column (3 $\times$ 100 mm, 3.5 μ m; Waters, Milford, MA); a linear gradient of mobile phase A:B (v/v) was used starting from 70:30 at 0 min to 5:95 in 9 min at a flow rate of 0.5 ml/min, where A was 5 mM ammonium acetate with 0.1% formic acid, pH 4.0, and B was acetonitrile with 0.1% formic acid. Micafungin was detected by fluorescence emission at 464 nm (excitation at 273 nm), and diclofenac was detected by UV at 280 nm; the retention times were 5.5 min for micafungin and 6.3 min for diclofenac. The method was linear for a micafungin concentration range of 0.1 to 5 μ M. Accuracy and precision of the method were 12 and 3.3% for 0.3 μ M and 0.2 and 2.8% for 2.5 μ M, respectively.

Data Analysis. IC_{50} values were determined using WinNonlin (version 4) software by nonlinear regression according to eq. 1:

$$E = E_{\max} \times \left[1 - \frac{C}{C + \text{IC}_{50}} \right] \quad (1)$$

Nonlinear regression analysis using WinNonlin was applied to determine the Michaelis-Menten uptake kinetics parameters (K_m and $V_{\max}$) and the kinetic parameter Cl_d for the nonsaturable process according to eq. 2:

$$v = \frac{V_{\max} \times C}{K_m + C} + \text{Cl}_d \times C \quad (2)$$

where v represents the experimental uptake rate and C the micafungin concentration. The saturable uptake rate was calculated using eq. 3 after subtracting the uptake at 4°C:

$$v = \frac{V_{\max} \times C}{K_m + C} \quad (3)$$

Saturable uptake clearance ($V_{\max}/K_m$) was added to the nonsaturable uptake clearance (Cl_d) to obtain total in vitro $\text{Cl}_{\text{uptake}}$, which was used to predict in

vivo $\text{Cl}_{\text{plasma}}$ (Table 2). The biliary excretion index (BEI) (percentage) and in vitro intrinsic biliary clearance, $\text{Cl}_{\text{biliary}}$ (milliliters per minute per milligram protein), were calculated with B-CLEAR technology (Qualyst, Inc., Research Triangle Park, NC) based on eqs. 4 and 5 (Liu et al., 1999a):

$$\text{BEI} = \frac{\text{Accumulation}_{\text{cells+bile}} - \text{Accumulation}_{\text{cells}}}{\text{Accumulation}_{\text{cells+bile}}} \times 100 \quad (4)$$

$$\text{Intrinsic } \text{Cl}_{\text{biliary}} = \frac{\text{Accumulation}_{\text{cells+bile}} - \text{Accumulation}_{\text{cells}}}{\text{AUC}_{\text{medium}}} \quad (5)$$

where $\text{AUC}_{\text{medium}}$ was determined as the product of the incubation time and extracellular concentration. The in vitro intrinsic $\text{Cl}_{\text{biliary}}$ and intrinsic $\text{Cl}_{\text{uptake}}$ (milliliters per minute per milligram protein) values were scaled to kilograms of body weight using the scaling values listed in Table 2. In the absence of albumin during in vitro incubations, unbound intrinsic $\text{Cl}_{\text{biliary}}$ (intrinsic $\text{Cl}'_{\text{biliary}}$) and unbound intrinsic $\text{Cl}_{\text{plasma}}$ (intrinsic $\text{Cl}'_{\text{plasma}}$) were assumed to correspond to in vitro intrinsic $\text{Cl}_{\text{biliary}}$ (eq. 5) and in vitro intrinsic $\text{Cl}_{\text{uptake}}$, respectively. The predicted in vivo $\text{Cl}_{\text{biliary}}$ and $\text{Cl}_{\text{plasma}}$ values were estimated according to the well stirred model of hepatic disposition (eqs. 6 and 7), assuming a micafungin blood/plasma concentration ratio of 1.

$$\text{Predicted } \text{Cl}_{\text{biliary}} = \frac{Q_H \times f_u \times \text{intrinsic } \text{Cl}'_{\text{biliary}}}{Q_H + f_u \times \text{intrinsic } \text{Cl}'_{\text{biliary}}} \quad (6)$$

$$\text{Predicted } \text{Cl}_{\text{plasma}} = \frac{Q_H \times f_u \times \text{intrinsic } \text{Cl}'_{\text{plasma}}}{Q_H + f_u \times \text{intrinsic } \text{Cl}'_{\text{plasma}}} \quad (7)$$

where Q_H represents the hepatic blood flow rate and f_u the fraction of unbound drug (Table 2).

Statistical Analysis. Statistical analysis of the data was performed using one-way analysis of variance followed by a Dunnett's post test to compare micafungin disposition in inhibitor-treated hepatocytes versus control. Levels ($p < 0.05$, $p < 0.01$, and $p < 0.001$) of statistically significant inhibition of micafungin uptake or efflux compared with control values are clarified in the figure legends.

Results

Micafungin Uptake in Sandwich-Cultured Hepatocytes. The 10-min mean $\pm$ S.D. cellular uptake of micafungin in day 1 SCRH and day 6 SCHH was 280 ± 14 pmol/mg protein/min (three different hepatocyte preparations in triplicate) and 198 ± 26 pmol/mg protein/min (two different donors in triplicate), respectively. Micafungin hepatocyte uptake was decreased by 70 to 80% compared with control values when incubations were performed at 4°C, a condition in which active transport processes are almost completely absent (Fig. 2). The cellular uptake of micafungin also was inhibited by 45 to 55% in the presence of Na^+ -free (choline) buffer, a treatment known to specifically affect Na^+ -dependent uptake (mediated by NTCP/Ntcp) of bile acids. In the presence of 100 μ M taurocholate (a substrate for NTCP/Ntcp), micafungin uptake was inhibited by 53 and 63% in SCRH and SCHH, respectively. As shown in Fig. 2, modest inhibition (by 20–25%) was observed with 10 μ M rifampin (inhibitor of the Na^+ -independent transporter family, OATP/Oatp).

The NTCP/Ntcp substrate taurocholate inhibited micafungin (10 μ M; 10 min; Plus buffer) uptake in SCRH in a concentration-dependent manner with an IC_{50} value of 73.7 ± 8.4 μ M (Fig. 3A). In contrast, taurocholate (2.5 μ M) uptake was inhibited by micafungin with an IC_{50} value of 138.4 ± 16.0 μ M (Fig. 3B).

Concentration-dependent uptake of micafungin in rat (Fig. 4A) and human (data not shown) hepatocytes could be described by eq. 2 (see *Materials and Methods*), reflecting a combination of a saturable (Michaelis-Menten) process and a linear, nonsaturable process. Kinetic parameters (Table 1) describing active micafungin uptake were 40 ± 5 μ M (K_m) and 814 ± 248 pmol/(min $\cdot$ mg protein) [$V_{\max}$] in human hepatocytes, whereas a K_m of 39 ± 6 μ M and a $V_{\max}$ of $591 \pm$

FIG. 2. Effect of inhibitors of hepatic drug transporters on micafungin uptake in sandwich-cultured human and rat hepatocytes. Uptake of micafungin (10 μ M, 10 min) in sandwich-cultured rat ($\square$) and human ($\blacksquare$) hepatocytes at 4 or 37°C in the absence and presence of rifampin, choline (Na^+ -free) buffer, or taurocholate is shown. Bars represent mean relative uptake (as a percentage of control at 37°C) $\pm$ S.D. of triplicate determinations. Absolute values for control uptake (100%) were 280 ± 14 pmol/mg protein/min in rat (three different hepatocyte preparations) and 198 ± 26 pmol/mg protein/min in human hepatocytes (two different donors). Statistical significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, compared with control uptake in each species.

24 pmol/(min $\cdot$ mg protein) were obtained in rat hepatocytes (Fig. 4A). The calculated intrinsic clearance (V_{max}/K_m) values for active micafungin uptake were 20.5 ± 2.2 and 15.2 ± 4.4 $\mu\text{L}/(\text{min} \cdot \text{mg protein})$ in human and rat hepatocytes, respectively. Corresponding values for Cl_{d} , reflecting linear, nonsaturable uptake kinetics, were estimated to be 3.3 ± 0.5 and 3.8 ± 0.3 $\mu\text{L}/(\text{min} \cdot \text{mg protein})$, respectively (Table 1).

The contribution of both active and passive mechanisms to micafungin uptake in hepatocytes is supported further by the observation that nonsaturable uptake occurred in the presence of active uptake inhibitors (Na^+ -free buffer mixed with rifampin) or at 4°C in SCRH. Saturable (active) uptake in SCRH was determined by subtracting the 4°C uptake profile from total uptake as shown in Fig. 4B. The nonlinear regression analysis of this active uptake component obeyed Michaelis-Menten kinetics similar to those generated from the approach shown in Fig. 4A and Table 1. The nonlinear regression analysis for the net uptake as a function of micafungin concentration using the Michaelis-Menten eq. 3 revealed comparable kinetic parameters of 49 μM for K_m and 853 pmol/(min $\cdot$ mg protein) for V_{max} , yielding a value of 17.4 $\mu\text{L}/(\text{min} \cdot \text{mg})$ for intrinsic clearance (V_{max}/K_m) associated with the active process (data not tabulated).

Micafungin Biliary Excretion in SCRH. Accumulation of micafungin in day 4 SCRH was measured in the absence and in the presence of $\text{Ca}^{2+}/\text{Mg}^{2+}$ in the extracellular medium. The BEI and $\text{Cl}_{\text{biliary}}$ were determined by taking into account the accumulation differences under these conditions and by using eqs. 4 and 5. As illustrated in Fig. 5A, the mean BEI for micafungin in rat hepatocytes was $42 \pm 7\%$, compared with a BEI of $60 \pm 16\%$ for the well known substrate taurocholate (data not shown); the BEI of taurocholate has been reported to be 50 to 80% in SCRH (Annaert et al., 2001; McRae et al., 2006).

Transporters Involved in Micafungin Biliary Excretion in SCRH. The biliary excretion of micafungin was inhibited by 90% with the Bsep inhibitor taurocholate (Marion et al., 2007) and by $\sim 50\%$ with GF120918 (10 μM), a known inhibitor for P-gp and BCRP at this concentration (Ahmed-Belkacem et al., 2006) (Fig. 5A). Coincubation with CDFDA (the diacetate ester of the Mrp2 substrate

FIG. 3. Concentration-dependent inhibition of micafungin uptake by taurocholate and vice versa in sandwich-cultured rat hepatocytes. Inhibition of micafungin (10 μM) uptake by taurocholate (A) and inhibition of taurocholate (2.5 μM) uptake by micafungin (B). IC_{50} values were determined using WinNonlin software. The solid line represents the best fit to the uptake data according to eq. 1. The data reflect micafungin uptake expressed as a percentage of the no inhibitor control and are plotted as a function of inhibitor concentration. The IC_{50} value for taurocholate inhibition of micafungin uptake was 73.7 ± 8.4 μM ($K_i = 61$ μM), whereas the IC_{50} value for micafungin inhibition of taurocholate uptake was 138.4 ± 16.0 μM ($K_i = 59$ μM). Each data point represents the mean $\pm$ S.D. of three separate measurements obtained in representative sandwich-cultured rat hepatocyte preparations.

carboxydichlorofluorescein) had no effect on the biliary excretion of micafungin. The possible role of Mrp2 in the biliary excretion of micafungin was examined further by determining the biliary excretion of micafungin in sandwich-cultured hepatocytes from TR(−/−) rats compared with wild-type rats. The results shown in Fig. 5B were obtained after preloading the hepatocytes with 10 μM micafungin followed by measuring micafungin efflux under standard conditions (efflux from cells to extracellular medium only) compared with $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free conditions (efflux from both cells and bile canaliculi to extracellular medium). These results illustrate that micafungin biliary excretion was the same in hepatocytes from wild-type and TR(−/−) rats. In addition, no effect of the Mrp2 inhibitor MK571 on micafungin biliary excretion could be observed. However, the efflux across the sinusoidal membrane ("cell") was slightly higher (20%) in TR(−/−) compared with wild-type hepatocytes ($p < 0.05$), whereas MK571 also showed a slight but statistically significant effect on the sinusoidal micafungin efflux in wild-type (13% decrease) and TR(−/−) (12% decrease) rat hepatocytes.

Micafungin Biliary Excretion in SCHH. After preloading human hepatocytes with micafungin, efflux corresponding to biliary excretion amounted to 31 to 55% of total efflux (Fig. 6). Nefazodone

FIG. 4. Micafungin uptake kinetics in sandwich-cultured rat hepatocytes. The values for initial velocity (10 min) of micafungin cellular uptake in day 1 culture were plotted against micafungin concentration in the extracellular medium. In A, the model (see eq. 2) incorporating a saturable (Michaelis-Menten) component and a linear component was fitted to the data using the Solver function in MS Excel. Each point represents the mean of three experimental values obtained in a representative hepatocyte preparation. In B, concentration-dependent uptake of micafungin in SCRH is shown for total uptake at 37°C in the absence (◆) or presence of rifampin in choline (Na^+ -free) buffer (■) and uptake at 4°C (▲); net saturable uptake (○) represents the difference between uptake at 37 and 4°C. The solid line represents the best fit of eq. 3 to the uptake data. Each point represents the mean of three measurements.

TABLE 1

Summary of micafungin saturable and nonsaturable uptake kinetics in SCRH and SCHH

Mean $\pm$ S.D. kinetic parameters reflecting micafungin uptake were obtained by nonlinear regression using a model incorporating both a saturable (Michaelis-Menten) component and a nonsaturable (linear) component. Taurocholate uptake clearance values in the two SCRH cultures used here were 22.5 ± 4.8 and 28.5 ± 2.6 $\mu\text{L}/(\text{min} \cdot \text{mg protein})$.

Kinetic Parameter	Rat	Human
K_m (μM)	39 ± 6	40 ± 5
$V_{\max}$ [$\text{pmol}/(\text{min} \cdot \text{mg protein})$]	591 ± 24	814 ± 248
Cl_d [$\mu\text{L}/(\text{min} \cdot \text{mg protein})$]	3.8 ± 0.3	3.3 ± 0.5
$V_{\max}/K_m$ [$\mu\text{L}/(\text{min} \cdot \text{mg protein})$]	15.2 ± 4.4	20.5 ± 2.2

significantly inhibited micafungin biliary excretion to approximately 10 to 25% of total efflux. A comparable effect was observed for taurocholate. A much less pronounced or no effect was observed when micafungin efflux was measured after coincubation with inhibitors for other canalicular transporters, including GF120918 for P-gp/BCRP, fumitremorgin C for BCRP, and MK571 for MRP2. The use of 10 μM

FIG. 5. Effect of inhibitors of bile canalicular transporters on micafungin (10 μM) biliary excretion in day 4 SCRH. A, hepatocytes were incubated with 10 μM micafungin for 10 min to determine accumulation of micafungin in cells + bile canaliculari (■, Plus buffer: contains $\text{Ca}^{2+}/\text{Mg}^{2+}$) or accumulation in cells only (□, Minus buffer: without $\text{Ca}^{2+}/\text{Mg}^{2+}$). Using B-CLEAR technology, biliary excretion of micafungin was calculated from the difference in accumulation (■) between the Plus and Minus buffer conditions. In vitro biliary excretion of micafungin was measured in the absence and presence of taurocholate (100 μM), GF120918 (GF918, 10 μM), or CDFDA (100 μM). Numbers [%] represent the BEI values calculated according to eq. 4. B, after loading sandwich-cultured hepatocytes prepared from wild-type (WT) or TR(-/-) rats with micafungin (10 μM) for 10 min, micafungin efflux was determined in Plus buffer [efflux from cell to extracellular medium occurs across sinusoidal membrane only (□)] and in Minus buffer [efflux from cell to extracellular medium occurs across sinusoidal and canalicular membranes (■)]. Incubations also were conducted in the presence of the MRP2 inhibitor MK571. Numbers (%) represent relative efflux to bile canaliculari as a percentage of total efflux. Statistical significance: #, $p < 0.05$; ##, $p < 0.01$ for biliary excretion, compared with control (A); *, $p < 0.05$ for efflux across the sinusoidal membrane, either in TR(-/-) compared to WT hepatocytes, or in hepatocytes treated with MK571 compared to the corresponding untreated hepatocytes [WT or TR(-/-)].

GF120918 (but not 1 μM GF120918) seemed to result in a reduced canalicular micafungin excretion, an effect that was statistically significant in two of three donors. Likewise, the BCRP inhibitor fumitremorgin C significantly inhibited micafungin biliary efflux in hepatocyte preparations from one of two donors. Consistent with the observation in SCRH, the MRP2 inhibitor MK571 did not influence biliary excretion of micafungin in human hepatocytes. However, a significant ($p < 0.05$) reduction in the efflux of micafungin across the sinusoidal membrane could be observed.

Estimation of Plasma and Biliary Clearance of Micafungin in Human and Rat. The intrinsic biliary clearance values, calculated on the basis of results from in vitro incubations of micafungin with SCRH and SCHH according to eq. 5 were 18.7 ± 5.1 $\mu\text{L}/(\text{min} \cdot \text{mg protein})$ in rat and 13.8 ± 8.5 $\mu\text{L}/(\text{min} \cdot \text{mg protein})$ in human. Intrinsic

FIG. 6. Effect of inhibitors of bile canalicular transporters on micafungin biliary excretion in three batches of sandwich-cultured human hepatocytes. After sandwich-cultured human hepatocytes were loaded with micafungin (10 μ M) for 10 min, micafungin efflux was determined in Plus buffer [efflux from cell to extracellular medium occurs across sinusoidal membrane only ($\square$)] and in Minus buffer [efflux from cell to extracellular medium occurs across sinusoidal and canalicular membranes ($\blacksquare$)]. Efflux also was measured in the presence of inhibitors as indicated; MK571, taurocholate, and nefazodone were used at 25, 100, and 25 μ M, respectively. Bars represent mean $\pm$ S.D. ($n = 3$) efflux of triplicate measurements in each batch. Numbers in parentheses indicate the percentage of biliary efflux relative to total efflux (from cell + bile) for each treatment. Statistical significance compared with control: *, $p < 0.05$ for the efflux across sinusoidal membrane; #, $p < 0.05$; ##, $p < 0.01$ for biliary excretion. GF918, GF120918; FTC, fumitremorgin C.

plasma clearance, calculated from in vitro uptake kinetics of micafungin (see *Materials and Methods*), was 19.0 μ L/(min $\cdot$ mg protein) in rat and 23.5 μ L/(min $\cdot$ mg protein) in human. These intrinsic clearance values were scaled to total body intrinsic clearance values using the scaling parameters shown in Table 2. In vivo clearance values were estimated from the micafungin f_u and Q_H in human and rat, along with the scaled intrinsic clearance values, assuming the well stirred model for hepatic disposition (see eqs. 6 and 7). Good agreement was found between observed in vivo plasma clearance values for micafungin in rat and human and the corresponding calculated values based on in vitro uptake clearance measurements. In contrast, at the level of biliary excretion, human in vivo biliary clearance of micafungin was well predicted on the basis of in vitro data, whereas in vivo rat biliary clearance was approximately 5-fold overpredicted.

Discussion

Biliary excretion has been reported to account for approximately 90% of micafungin clearance (Hebert et al., 2005a). The relatively large molecular weight of this antifungal compound and its negatively charged sulfate function at physiological pH strongly suggest the involvement of drug transporters both in the hepatic uptake and biliary excretion of micafungin. Nevertheless, very little is known regarding the possible role of transporters in hepatobiliary elimination of micafungin in rats (Abe et al., 2008b), and no information is available in humans. In contrast, for another echinocandin, caspofungin, the active transport mechanisms possibly mediating its hepatic uptake have been investigated in experiments that used HeLa cells heterologously expressing the hepatic OATP isoforms (Sandhu et al., 2005). These uptake transporters have been suggested to play key roles in the hepatic elimination of caspofungin. It is also important to understand the role that transport proteins play in the sinusoidal uptake and canalicular excretion of micafungin.

To obtain this information in the present study, we used sandwich cultures of rat and human hepatocytes. Prior investigations have demonstrated the utility of sandwich-cultured hepatocytes to better understand the mechanisms governing hepatic uptake and biliary excretion of endogenous and exogenous compounds (Chandra et al., 2001; Annaert and Brouwer, 2005; Kemp et al., 2005; McRae et al., 2006). This in vitro model allows simultaneous assessment of hepatic uptake and biliary excretion, and the relative contributions of each process to overall hepatobiliary disposition can be derived.

Our in vitro data on micafungin uptake show that, like caspofungin, transporters of the OATP/Oatp family in both human and rat hepatocytes mediate micafungin uptake. However, OATPs/Oatps played a modest role compared with the Na^+ -dependent micafungin uptake, which is a process mediated by NTCP/Ntcp. Micafungin uptake was inhibited most profoundly by taurocholate or Na^+ depletion in both SCRH and SCHH. In SCRH, the concentration-dependent inhibition of taurocholate uptake by micafungin ($\text{IC}_{50} = 138 \mu\text{M}$) (Fig. 3B) was evaluated; on the basis of the Cheng and Prusoff (1973) equation, the estimated K_i for inhibition of taurocholate uptake by micafungin was 59 μM . A comparable value ($K_i = 61 \mu\text{M}$) was obtained for inhibition of micafungin uptake by taurocholate. Taken together, these data support the hypothesis that NTCP/Ntcp plays a predominant role in micafungin uptake in both rat and human hepatocytes. This result is somewhat remarkable because NTCP is still thought to be quite specific for mediating hepatic uptake of bile acids. Indeed, only a few nonbile acid substrates for NTCP/Ntcp have been reported, including estrone-3-sulfate (Schroeder et al., 1998; Kullak-Ublick et al., 2000) and rosuvastatin (Ho et al., 2006). The structure of these previously identified nonbile acid substrates, as well as that of micafungin, features a sulfate or sulfonamide functional group.

Using B-CLEAR technology (Liu et al., 1999a; Hoffmaster et al., 2004; Ghibellini et al., 2007), we showed pronounced in vitro biliary excretion of micafungin, both in SCRH (by accumulation experiments) (Fig. 5) and in SCHH (by efflux experiments after preloading hepatocytes; see *Materials and Methods*) (Fig. 6). The suitability of the three batches of SCHH used in the present study to determine biliary excretion of micafungin was assessed by measuring the biliary excretion index of the established substrate taurocholate, which was found to be $62 \pm 15\%$. This result compares well with the previously reported BEI of taurocholate in sandwich-cultured human hepatocytes of 56% (Ghibellini et al., 2007). In SCRH, incubation of micafungin with 100 μM taurocholate resulted in a reduction in micafungin accumulation under standard conditions to the same levels as observed in Ca^{2+} -free conditions (Fig. 5A). Taurocholate was also the

TABLE 2

Calculation of in vivo plasma and biliary clearance of micafungin based on in vitro data obtained in rat and human hepatocytes

In vitro CI values and in vivo predicted CI values represent mean $\pm$ S.D. ($n = 2$ – 3 batches of hepatocytes). Intrinsic CI values were generated as described under *Materials and Methods*. Intrinsic uptake clearance was calculated by combining the saturable and nonsaturable clearance values that are listed in Table 1. Intrinsic biliary clearance was determined using B-CLEAR technology (Liu et al., 1999). Scaled intrinsic clearance and predicted in vivo clearance were calculated as described by Abe et al. (2008c) using eq. 6 (well stirred model of hepatic drug clearance). In vivo biliary clearance in humans was obtained by multiplying total clearance with the fraction of a micafungin dose eliminated in bile (based on a human mass balance study with radiolabeled micafungin; 71% of the eliminated dose was recovered in bile/feces, whereas only trace amounts of metabolites were detected).

Physiological Parameters and Scaling Factors	Rat	Human
f_o	0.003 ^a	0.004 ^b
Hepatic protein content mg protein/g of liver	200 ^c	71 ^d
Relative liver weight g liver/kg b.wt.	40 ^c	26 ^d
Q_H [ml/(min $\cdot$ kg)]	40 ^c	21 ^{d,f}
In vivo plasma clearance [ml/(min $\cdot$ kg)]	1.25 ^g	0.229 ^h
In vitro intrinsic uptake clearance [ml/(min $\cdot$ mg protein)]	0.0190 $\pm$ 0.0044	0.0235 $\pm$ 0.0016
Intrinsic uptake clearance [ml/(min $\cdot$ kg)]	152.4 $\pm$ 35.3	43.38 $\pm$ 2.95
Calculated in vivo plasma clearance [ml/(min $\cdot$ kg)]	0.447 $\pm$ 0.104	0.175 $\pm$ 0.012
In vivo biliary clearance [ml/(min $\cdot$ kg)]	0.085 ^g	0.16 ^e
In vitro intrinsic biliary clearance [ml/(min $\cdot$ kg)]	0.019 $\pm$ 0.005	0.014 $\pm$ 0.009
Intrinsic biliary clearance [ml/(min $\cdot$ kg)]	149.3 $\pm$ 41.1	25.54 $\pm$ 15.69
Calculated in vivo biliary clearance [ml/(min $\cdot$ kg)]	0.438 $\pm$ 0.120	0.101 $\pm$ 0.062

^a Data from Abe et al. (2008a).

^b Data from S. B. Yanni, P. B. Smith, D. K. Benjamin, P. Augustijns, D. R. Thakker, and P. P. Annaert, manuscript submitted for publication.

^c Data from Abe et al. (2008c).

^d Data from Ghibellini et al. (2007).

^e Data for Mycamine (micafungin sodium) from Astellas Pharma US Inc. [full prescribing information (§12.3. Pharmacokinetics), <http://www.astellas.us/docs/mycamine.pdf>].

^f Data from Björkman (2005).

^g Data from Abe et al. (2008b).

^h Data from Wiederhold and Lewis (2007).

most effective inhibitor of micafungin biliary excretion in SCHH on the basis of taurocholate-mediated inhibition of micafungin canalicular efflux from preloaded hepatocytes (Fig. 6). Our in vitro biliary excretion data with micafungin in SCRH and SCHH support the primary involvement of BSEP/Bsep in micafungin biliary excretion. In SCHH, inhibition of micafungin biliary excretion by the BSEP inhibitor nefazodone (Kostrubsky et al., 2006) provided further evidence for the role of BSEP. The inhibitory effects observed with the mixed P-gp/BCRP inhibitor GF120918 and the selective BCRP inhibitor fumitremorgin C in SCHH suggests that BCRP, but not P-gp, plays some role in micafungin biliary excretion. The latter observation is consistent with a previous report that micafungin is not a substrate for P-gp (Sakaeda et al., 2005).

In SCRH, we specifically investigated the possible role of Mrp2 in the biliary excretion of micafungin by conducting in vitro biliary excretion experiments in sandwich-cultured hepatocytes from TR(–/–) rats, which are deficient in functional Mrp2. In vitro micafungin biliary excretion in these mutant rats was unaltered compared with that in wild-type rats. Furthermore, in vitro micafungin biliary excretion was unaffected by the presence of the Mrp2 substrate carboxydiclorofluorescein or the Mrp2 inhibitor MK571. These data suggest that either Mrp2 does not play a significant role in micafungin biliary excretion in rats, or that another canalicular protein is able to completely compensate for the absence of Mrp2. Our observation is inconsistent with the recent in vivo study by Abe et al. (2008b), who reported that micafungin biliary clearance was reduced (by approximately 60%) in TR(–/–) rats, suggesting an important role of Mrp2 in the biliary excretion of micafungin in normal rats in vivo. However, apart from undetectable Mrp2 levels, Abe et al. (2008c) showed that Bcrp expression was reduced markedly in TR(–/–) rats, indicating that reduced biliary drug clearance in these mutant rats is not necessarily due to Mrp2 involvement. Alternatively, discrepancies between our in vitro data and previous in vivo data on the involvement of Mrp2 in biliary excretion of micafungin also could be caused by altered transporter expression levels in cultured hepatocytes compared with the liver in vivo. It is also noteworthy that the expression and function of Mrp3 are enhanced in TR(–/–) rats compared with wild-type rats (Xiong et al., 2002). Thus, the decrease in biliary clearance of mica-

fungin in TR(–/–) rats (Abe et al., 2008b) also might be caused by increased Mrp3-mediated sinusoidal efflux rather than decreased Mrp2-mediated canalicular efflux of micafungin. In the present study, we also observed slight but significant reductions in sinusoidal micafungin efflux upon coinubation with the Mrp2/Mrp3 inhibitor MK571, which further supports that a sinusoidal efflux transporter (such as Mrp3) but not Mrp2 (localized at the canalicular membrane) plays a role in micafungin disposition in SCRH. Chemical inhibition experiments with MK571 in SCHH (Fig. 6) also confirm that in human liver MRP2 is not extensively involved in micafungin biliary excretion or that other canalicular protein(s) are able to compensate for and maintain biliary excretion of micafungin. Given the very limited overall metabolism of micafungin (Hebert et al., 2005a; Astellas Pharma Inc., 2006) as well as the absence of any conjugates (phase II metabolites are typical MRP2 substrates), it is also unlikely that MRP2 plays a significant role in elimination of micafungin metabolites. Reduced micafungin efflux across the sinusoidal membrane of SCHH was observed in the presence of MK571. This may support a role for a sinusoidal efflux transporter, such as MRP3, in micafungin disposition in SCHH. However, it should be noted that the effect of MK571 on micafungin efflux also may be related to decreased accumulation of micafungin during the loading phase. Recent reports (Matsson et al., 2009) suggest poor specificity of MK571, whereby transporters other than MRPs, such as OATP (Janneh et al., 2010), were affected by MK571. Nevertheless, given the rather limited role of OATPs in micafungin uptake, it remains unlikely that MK571 would cause a substantial reduction in micafungin uptake during the loading phase.

In the same in vivo rat study involving micafungin, Abe et al. (2008b) showed that the systemic clearance of micafungin in normal rats was reduced after intravenous cyclosporine A administration. Whereas cyclosporine A is an inhibitor of MRP2/Mrp2, it is also an inhibitor of NTCP (Mita et al., 2006), OATPs/Oatps (Shitara et al., 2009), and BSEP/Bsep (Mita et al., 2006). Accordingly, in the context of our in vitro data, cyclosporine A is likely to decrease micafungin systemic clearance by inhibiting Ntcp and/or Oatp-mediated uptake of micafungin and/or by inhibiting Bsep-mediated (and Bcrp-mediated) biliary excretion in rats. However, there is little evidence for Mrp2

FIG. 7. Schematic illustration of transporter-mediated hepatobiliary disposition of micafungin. Hepatic micafungin uptake occurs by $\text{NTCP} > \text{OATP}$, whereas secretion across the canalicular hepatocyte membrane is mediated by $\text{BSEP} > \text{BCRP}$. MRP3 appears to be responsible for the micafungin efflux across the sinusoidal hepatocyte membrane.

being involved in this drug interaction, or in micafungin hepatobiliary disposition in rats.

In human liver, the role of NTCP/BSEP in the hepatobiliary elimination of micafungin can explain observed clinical interaction data reported by Hebert et al. (2005a) with cyclosporine A, which increased micafungin plasma AUC; however, the underlying mechanism has not been fully described. Our *in vitro* data support the inhibition of NTCP and/or BSEP by cyclosporine A (as previously described by Mita et al., 2006) as a primary mechanism to explain this clinical drug interaction. Another clinical implication of our findings is that extensive micafungin biliary excretion, mediated by BSEP, is responsible for its therapeutic success in the treatment of candidal cholecystitis (Maruyama et al., 2009). This finding also suggests that knowledge of transporter-mediated drug disposition mechanisms could provide a rational basis for establishing targeted drug delivery to specific tissues.

Comparison of our *in vitro* data for rat and human hepatocytes reveals a remarkable agreement between both species when the transporters involved in micafungin hepatobiliary disposition are considered. This observation contrasts with the pronounced interspecies differences that generally have been reported, not only for drug-metabolizing enzymes but also for hepatic drug transporters involved in drug elimination (Leslie et al., 2007) and interactions (Ye et al., 2010). To assess whether this qualitative species similarity in micafungin disposition also is reflected at the quantitative level, *in vivo* plasma and biliary clearance of micafungin in rats and humans was calculated based on the *in vitro* uptake and biliary excretion data obtained in sandwich-cultured rat and human hepatocytes (Table 2). There was general agreement between the observed plasma clearance values and those predicted based on *in vitro* hepatic uptake data. In contrast, *in vivo* biliary clearance values in humans were well predicted by *in vitro* biliary excretion data obtained in SCHH but were overpredicted in the rat. This may be due to the existence of metabolic clearance pathways for micafungin in rat liver *in vivo*, the functionality of which may not be fully reflected in day 4 SCRH. Reduced drug-metabolizing enzyme activities in SCRH compared with *in vivo* levels have been reported previously (Liu et al., 1999a). Comparison of the *in vivo* biliary clearance values with *in vivo* plasma clearance values (Table 2) suggests that biliary excretion of micafungin rather

than hepatic uptake is rate-limiting for its hepatic elimination in humans.

In conclusion, using sandwich-cultured rat and human hepatocytes, we have characterized the micafungin hepatic uptake kinetics and identified NTCP/Ntcp and BSEP/Bsep as important mediators of micafungin hepatocyte uptake and biliary excretion, respectively, in both species. Figure 7 depicts the various mechanisms underlying the hepatobiliary disposition of micafungin. It remains to be investigated to what extent the sharing of NTCP/BSEP between micafungin and bile acids may be responsible for side effects in patients receiving micafungin therapy. In this context, it is noteworthy that a warning for hepatotoxicity has been included in the micafungin label, and that a recent case report (Anonymous, 2009) supports a possible association between hepatotoxicity and the use of micafungin. More pronounced inhibition of BSEP (over NTCP) by micafungin theoretically could lead to cholestasis, and this mechanism has been associated with the hepatotoxicity of several drugs including bosentan (Leslie et al., 2007). As of today, however, there are no reports that micafungin causes cholestasis in the clinic, which might be explained by the fact that micafungin inhibits NTCP to approximately the same extent as BSEP. Reported total plasma concentration maxima of micafungin in humans are in the range of 5 to 8 μM , whereas protein binding is very high (>99.5%). Given a K_i value of 59 μM for inhibition of taurocholate uptake by micafungin in rat liver, and assuming a comparably high K_i applies for inhibition of human NTCP, the interference of micafungin with bile acid uptake may not be clinically relevant. Furthermore, coadministration of micafungin with other drugs (e.g., cyclosporine A) that affect NTCP/BSEP with micafungin also will determine the clinical relevance of potential interactions between micafungin and bile acid homeostasis and elimination. It is clear that future *in vitro* experiments in sandwich-cultured hepatocytes using clinically relevant drug combinations may support the rational prevention of possible drug interactions and toxicity in patients taking multiple drugs including micafungin.

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