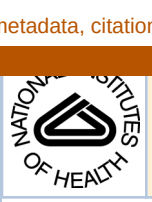



Published in final edited form as:

*Biopharm Drug Dispos*. 2011 May ; 32(4): 222–232. doi:10.1002/bdd.752.

# Higher clearance of micafungin in neonates compared to adults: role of age-dependent micafungin serum binding

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## Abstract

Micafungin, a new echinocandin antifungal agent, has been widely used for the treatment of various fungal infections in human populations. Micafungin is predominantly cleared by biliary excretion and it binds extensively to plasma proteins (>99.5%). Micafungin body weight-adjusted clearance is higher in neonates than in adults, but the mechanisms underlying this difference are not understood. Previous work had revealed the roles of sinusoidal uptake ($Na^+$-taurocholate co-transporting peptide, NTCP; organic anion transporting polypeptide, OATP) as well as canalicular efflux (bile salt export pump, BSEP; breast cancer resistance protein, BCRP) transporters in micafungin hepatobiliary elimination. In the present study, the relative protein expression of hepatic transporters was compared between liver homogenates from neonates and adults. Also, the extent of micafungin binding to serum from neonates and adults was measured *in vitro*. The results indicate that relative expression levels of NTCP, OATP1B1/3, BSEP, BCRP, and MRP3 were similar in neonates and in adults. However, micafungin fraction unbound ($f_u$) in neonatal serum was about 8-fold higher than in adult serum (0.033 ± 0.012 *versus* 0.004 ± 0.001, respectively). While there was no evidence for different intrinsic hepatobiliary clearance of micafungin between neonates and adults, our data suggest that age-dependent serum protein binding of micafungin is responsible for its higher clearance in neonates compared to adults.

### Keywords

micafungin; pediatric drug disposition; serum binding; clearance prediction; antifungal

## Introduction

Micafungin is a new semi-synthetic echinocandin-type antifungal drug that has been widely used for the treatment of various fungal infections in human populations. Pharmacokinetic studies with $^{14}$C-micafungin determined that approximately 90% of the plasma clearance occurs via biliary elimination of the parent drug [1], whereas urinary excretion is a minor elimination route in human and rat [2–4]. Like caspofungin and anidulafungin, micafungin

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is a peptide-like compound. Due to the presence of a sulfate group, micafungin is negatively charged at physiological pH (see Figure 1). Micafungin displays high plasma protein binding (>99%) in humans and animals [4].

Pharmacokinetic parameters of micafungin were determined in several human age groups: neonates < 1 month old, children <8 years of age, adolescents, and adults [5,6]. Micafungin was found to have a higher body weight-adjusted clearance in neonates than older groups. The necessity to use higher body-weight adjusted doses in neonates in order to achieve therapeutic plasma concentrations was recently confirmed by a population pharmacokinetic analysis [7]. As of today, no studies have been conducted to elucidate the cause of this age-difference in the disposition of micafungin in humans.

In order to obtain better insight in the age-dependent disposition of micafungin, it was crucial to first understand the clearance mechanisms in the adult population. In this context, we have recently conducted *in vitro* transport studies with micafungin in sandwich-cultured human hepatocytes (SCHH) [8], which revealed the following: a) the sinusoidal uptake transporter $Na^+$-taurocholate co-transporting peptide (NTCP, *SLC10A1*) and the canalicular efflux transporter Bile Salt Export Pump (BSEP, *ABCB11*) were found to mediate the hepatobiliary disposition of micafungin; b) Breast Cancer Resistance Protein (BCRP, *ABCG2*), Multidrug Resistance Associated Protein 3 (MRP3, *ABCC3)*, and Organic Anion Transporting Polypeptide (OATP, *SLCO*) were found to modestly associate with the disposition of micafungin in SCHH; c) by using B-CLEAR® technology (Qualyst Inc., Research Triangle Park, NC) [9–11], *in vivo* plasma and biliary clearance of micafungin were calculated based on the *in vitro* data obtained in SCHH and found to be in agreement with the clinical data. Because of ethical considerations, it is almost impossible to obtain fresh liver tissue from neonates suitable for establishing SCHH, which would support investigating transporter-mediated disposition of micafungin and predicting *in vivo* hepatobiliary clearance like in adults. However, it was possible to obtain frozen liver tissue samples from neonates (and adults) to support determining the protein expression of hepatic drug transporters. In addition, because protein binding is known to influence drug distribution and drug clearance, and because it is variable as a function of age [12–15], the extent of micafungin serum binding in neonates was determined *in vitro*.

In this study, we calculated *in vivo* hepatobiliary clearance of micafungin in neonates based on: *a*) the *in vitro* intrinsic clearance in adults as determined previously [8]; *b*) scaling factors applicable for human neonatal liver; and *c*) micafungin fraction unbound in serum of neonates. We showed that the calculated *in vivo* plasma and biliary clearance in neonates are in agreement to those observed in clinic. Our data provide evidence for the mechanism underlying higher clearance of micafungin in neonates compared to adults.

## Materials and Methods

### Chemicals and Reagents

Micafungin–sodium was a gift from Dr. John Perfect at Duke University. Serum samples from neonates or adult (n=6/group) were provided by Dr. Daniel Benjamin at Duke Center Research Institute. Frozen liver from neonates and adults (5 samples per group) were obtained from the Comparative Human Tissue Network (CHTN) (Columbus, OH) under an approved UNC-Chapel Hill Institutional Review Board protocol. NuPAGE 4–20% Bis-Tris gel and all other gel electrophoresis and Western blot reagents and supplies were purchased from Invitrogen (Carlsbad, CA). Primary antibodies against human BSEP [*ABCB11*] (F-6), NTCP [*SLC10A1*] (M-130) and OATP1B1 [*SLCO1B1*] (and OATP1B3 [*SLCO1B3*] to a lesser extent) (N-16) were purchased from Santa Cruz (Santa Cruz, CA), while those against MRP3 [*ABCC3*] (M3II-9), BCRP [*ABCG2*] (BXP-53), and GAPDH were purchased from

Alexis (San Diego, CA). Horseradish peroxidase-conjugated secondary antibody suitable for each primary antibody and the chemi-luminescent substrate SuperSignal® West Dura were purchased from Pierce Chemical (Rockford, IL).

### Protein Expression of Drug Transporters in Liver Tissues from Neonates and Adults

Frozen liver tissues from neonates (postnatal age 0–1 month), and from adults (older than 18 years) were homogenized in 1.15% KCL buffer, pH 7.4 (1:3, w/v) and crude homogenates were prepared after centrifugation at 800 rpm for 3 min. Protein concentration in crude liver homogenates was determined by the bicinchoninic acid method against bovine serum albumin standard, and homogenates at 1mg/ml were prepared in sample buffer containing a protease inhibitors cocktail (Sigma-Aldrich, St. Louis, MO). Western blot analysis for the expression of transport proteins was performed by separating the transport proteins in liver homogenate samples loaded on SDS page gel (20 μl/well) with gel electrophoresis and the proteins were then transferred to nitrocellulose membranes according to the vendor's instructions (Invitrogen, Carlsbad, CA). Primary antibodies against human NTCP, BSEP, OATP1B1/3, MRP3, BCRP, GAPDH were appropriately diluted (1:200, 1:200, 1:200, 1:200, 1:500, and 1:4000, respectively) and incubated with the membrane for 2 hr at 37°C. Horseradish peroxidase-conjugated secondary antibody suitable for each primary antibody was cross-reacted with the transporter protein-primary antibody complex; immunoreactive protein bands were detected using a Bio-Rad VersaDoc imaging system (Bio-Rad, Hercules, CA). For quantitative determination of protein expression, three density measurements of each band corresponding to BSEP, OATP1B1/3, MRP3, BCRP, GAPDH (molecular weight 190 kDa, 90 kDa, 170 kDa, 70 kDa, 35 kDa, respectively) were determined and the mean density was normalized to the mean density of the GAPDH band (used to adjust expression based on actual loaded protein samples). Relative expression of the four transporters to GAPDH in each liver homogenate sample in the neonatal or adult group was determined for each of the five liver homogenate samples per group. The mean was expressed per kg body weight based on the following scaling parameters: 35 and 70 mg protein/gram liver in neonates and adult (experimentally determined), respectively; 120 g and 1500 g average liver weight in neonates or adult (data listed by Björkman [16]), respectively; 3 kg and 70 kg average body weight in neonates and adult (data listed by Björkman [16]), respectively. The relative protein expression in neonates was expressed as percent of the mean expression in adult samples.

### Micafungin Serum Fraction Unbound in Neonates and Adults

Micafungin fraction unbound in serum from human neonates or adults was determined by equilibrium dialysis according to a modified method described by Kalvass and Maurer [17]. Briefly, Spectra/Por® 2 dialysis membranes (Spectrum Laboratories Inc., Rancho Dominguez, CA) were conditioned in water for 15 min, followed by 30% ethanol for 15 min, and sodium phosphate buffer (100 mM, pH 7.4) for 15 min. Serum samples from neonates (prenatal age 23–40 weeks of gestation; postnatal age 1–52 days; body weight 0.6–3.5 kg) (n=6) or adults (n=6) were spiked with micafungin (10 μM) and 70 μl aliquots of each sample were loaded into a 96-well equilibrium dialysis apparatus (HTDialysis, Gales Ferry, CT) and dialyzed against an equal volume of sodium phosphate buffer (100 mM, pH 7.4). The 96-well equilibrium dialysis apparatus was incubated for 4.5 hr with orbital shaking (100 rpm) at 37°C. These equilibrium dialysis conditions were found to be sufficient for micafungin to reach equilibrium. Aliquots from dialyzed samples (serum side and buffer side) were processed and the concentrations of extracted micafungin in both serum and buffer matrices were determined based on calibration curves of micafungin prepared in the corresponding matrix. Micafungin $f_u$ was determined according to Equation 1.

## Analytical Method

An HPLC method was developed to separate micafungin and diclofenac (internal standard) using a C8 XTerra® column (3 × 100 mm, 3.5μ Waters); a linear gradient of mobile phase A:B (v/v) was used starting from 70:30 at 0 min to 5:95 in 9 minutes 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), while 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 0.1–5 μM. Accuracy and precision of the method were 12% and 3.3% for 0.3 μM and 0.2% and 2.8% for 2.5 μM, respectively.

## Data Analysis

Micafungin $f_u$ was determined from the following equation:

$$f_u = \frac{[micafungin_{buffer}]}{[micafungin_{plasma}]} \quad \text{(Equation 1)}$$

The $f_u$ values in six neonates were plotted against the age of gestation at birth (in weeks) or against postnatal age at birth (in days) of each subject.

*In vivo* micafungin plasma and biliary clearance values were predicted based on previously obtained *in vitro* data [8], and the serum binding data obtained in the present study. The *in vitro* intrinsic biliary clearance (ml/min/mg protein) and *in vitro* intrinsic accumulation clearance were first scaled to kg body weight using the parameters listed in Table 1 as published by Björkman *et al*. [16]. In the absence of albumin during *in vitro* incubations [8], unbound intrinsic biliary clearance (intrinsic $Cl'_{biliary}$) and unbound intrinsic plasma clearance (intrinsic $Cl'_{plasma}$) were assumed to correspond to *in vitro* intrinsic biliary clearance and *in vitro* intrinsic accumulation clearance, respectively. The calculated *in vivo* biliary clearance ($Cl_{biliary}$) and *in vivo* plasma (accumulation) clearance ($Cl_{plasma}$) values were calculated according to the well-stirred model of hepatic disposition, assuming a micafungin blood/plasma concentration ratio of 1:

$$\text{Calculated } in\ vivo\ Cl_{biliary} = \frac{Q_H \times f_u \times \text{intrinsic } Cl'_{biliary}}{Q_H + f_u \times \text{intrinsic } Cl'_{biliary}} \quad \text{(Equation 2)}$$

$$\text{Calculated } in\ vivo\ Cl_{plasma} = \frac{Q_H \times f_u \times \text{intrinsic } Cl'_{plasma}}{Q_H + f_u \times \text{intrinsic } Cl'_{plasma}} \quad \text{(Equation 3)}$$

where $Q_H$ represents the hepatic plasma flow rate. The $Q_H$ values in neonate and adult are listed in Table 1.

## Statistical Analysis

An unpaired *t*-test (assuming unequal variance) was performed to determine the significance of the difference in micafungin $f_u$ between neonates and adults.

## Results

### Protein Expression of Drug Transporters in Liver Tissues from Neonates and Adults

The immunostaining bands corresponding to NTCP, BSEP, BCRP, MRP3, and the hepatic isoforms belonging to the OATP1B subfamily in five neonatal liver homogenate samples (N1–N5) and in five adult liver homogenate samples (A1–A5) are shown in the upper part of Figure 2. As illustrated in the lower panel of Figure 2, the protein expression levels of these transporters in neonates, relative to GAPDH and expressed per kg body weight, were between 84 and 115% of adult levels. No statistically significant differences were observed in transporter expression levels between neonates and adults.

### Serum Fraction Unbound of Micafungin in Neonates and Adults

Using equilibrium dialysis, micafungin $f_u$ was determined as function of time and drug concentration (data not shown). In neonates, the mean $f_u$ from six different neonatal samples conducted at three different days was found to be 0.033 ± 0.017 (96.7% bound) compared to a mean $f_u$ value of 0.004 ± 0.003 (99.6% bound) in adult (Figure 3). The difference of $f_u$ between neonates and adults is statistically significant ($P < 0.001$). Figure 4 illustrates the individual $f_u$ values in neonates along with the postgestational age at birth as well as the postnatal age. The highest $f_u$ was observed for the full term subjects 1 and 3 (40 weeks postgestation), while lower $f_u$ values were found for premature subjects 2 and 4 (24 and 31 weeks of gestation, respectively). The lowest micafungin $f_u$ was observed for subject 2, which is not only the most premature, but also the oldest (52 days after birth) baby.

These results indicate that micafungin $f_u$ in neonate serum is higher than in adult serum. These results also indicated that, while there appears to be a weak relationship between postgestational age at birth and $f_u$, there is no evidence for a relationship between postnatal age in neonates and $f_u$.

### Prediction of *in vivo* Plasma and Biliary Clearance of Micafungin in Neonates

*In vitro* intrinsic accumulation Cl and *in vitro* intrinsic biliary Cl of micafungin have recently been determined using sandwich-cultured human hepatocytes from adult liver donors [8], and were 23.5 ± 1.6 and 14.0 ± 9.0 μl/min/mg, respectively. To estimate *in vitro* intrinsic clearance values for micafungin in neonates, *in vitro* intrinsic clearance values of adults were used, given that expressions of drug transporters associated with the clearance of micafungin in neonates and adults were comparable (Figure 2). As illustrated in Table 1, intrinsic clearance values were scaled to *in vivo* intrinsic clearance using neonate-based scaling factors as reported [16,18]. *In vivo* intrinsic clearance values were then used to calculate *in vivo* clearance values based on the well-stirred model (Equation 2 and Equation 3), taking into account micafungin $f_u$ (as determined in the present study) as well as adult/neonatal values for $Q_H$. The results shown in Table 1 illustrate a good agreement between the observed and calculated plasma clearance values. In addition, the calculated clearance values in neonates were 3-fold higher than in adults as it has been observed in clinic.

## Discussion

Micafungin, which is used to treat life-threatening fungal CNS infection in neonates and young infants, is mainly cleared into the bile [1]. Surprisingly, the clearance of micafungin in neonates, the human population generally displaying immature drug metabolizing enzymes and drug transporter function, is reported to be three times that of adults. Because it undergoes biliary clearance, we hypothesized that the higher clearance of micafungin in neonates might be due to the higher drug uptake and/or secretion by sinusoidal and canalicular liver transporters. The transporters that mediate the uptake and secretion of

micafungin were identified in our previous *in vitro* mechanistic study using human sandwich-cultured hepatocytes prepared from fresh liver of adult donors [8]. These *in vitro* data confirmed the unique utility of this *in vitro* model system in exploring the mechanisms of hepatic uptake and biliary excretion of endogenous and exogenous compounds and in predicting *in vivo* biliary clearance of drugs [9–11,19–22]. In our previous study, we have indeed reported that in *in vitro* hepatic accumulation of micafungin in adult human hepatocytes occurred mainly by transporter-mediated mechanisms. The uptake of micafungin into the hepatocytes was mediated by NTCP ≫ OATP, whereas the biliary excretion involved the canalicular transporters BSEP > BCRP. The results also suggested a role for the sinusoidal efflux transporter MRP3, in the hepatic disposition of micafungin.

Unfortunately, because of ethical considerations, it is nearly impossible to obtain human neonatal liver tissue suitable for establishing hepatocyte cultures. Therefore, we considered another approach to obtain better insight into the age-dependent clearance of micafungin. By measuring the protein expression of NTCP, BSEP, BCRP, MRP3 and both hepatic OATP1B isoforms in neonatal versus adult liver homogenates, we pursued to make a semi-quantitative assessment regarding the possible age-dependent activity of these transporters. A few studies on the ontogeny of transporter expression and function have indeed demonstrated that transporter protein expression levels correlate with function. For example, in a developmental mouse model, Pinto *et al*. [23] showed that digoxin accumulation in brain, kidney, and liver agreed with the expression level of the Mdr1a gene encoding for the P-gp efflux transporter in an age-dependent manner. Goralski *et al*. [24] have shown an agreement between the expression and function of transporters by demonstrating that deficient Mdr1a/b expression in neonatal mouse brain resulted in enhanced accumulation of digoxin and cyclosporin in neonatal mice compared to adult mice. As mentioned earlier, we have previously shown that micafungin is predominantly relying on the bile salt transporters NTCP and BSEP for its hepatobiliary elimination. At the uptake level, our data support comparable expression of the main micafungin transporter NTCP between neonates and adults. Previous work by Hardikar *et al*. [25] in rats, indicated that Ntcp protein was expressed as early as postnatal day 1, and the expression levels on day 1 and day 7 (equivalent to 1 month human neonate) postnatal liver were also comparable to those in adults. Furthermore, as shown in this study (see Figure 2), also BSEP exhibits comparable expression in neonates and in adults. This result was consistent with previous data on quantitative expression of BSEP and other canalicular transport proteins in human fetal, pediatric and adult livers [26,27]. In these studies, the authors reported that BSEP protein was expressed in fetus and pediatric human liver at the same level as adult, however, in fetus, the localization of BSEP was found to be at both the canalicular and cytosolic locations. In general, very limited information on the ontogeny of drug transporters in human, not only at the functional level, but also at the expression level has been obtained. Studies on the age-dependent expression and localization of MDR1/P-gp in human brain [28] and small intestine [29] are exceptions. In the rat, Gao *et al*. observed that the canalicular transporter Bsep was detected at the canalicular membrane before birth but reached adult expression levels by postnatal day 12 (day 7 in neonatal rat is equivalent to neonate in human) [30]. The authors indicated that by day 12, hepatocytes were fully polarized and localization of transporters (at either canalicular or sinusoidal membranes) was comparable to what was observed in adult hepatocytes. They also reported that the development of polarized hepatocyte membranes and expression of major bile salt transporters Bsep and Ntcp occurs shortly before birth and that correlated with the development of the enterohepatic circulation of bile salts. Therefore, full development of this excretion route early in neonatal life indicates that age-dependent expression (and thus function) of these transporters may not explain the age-dependent clearance of micafungin. It follows that the similarity in the expression of these transporters between neonates and adults can confidently be used to quantitatively estimate micafungin hepatic clearance in

neonates, based on the *in vitro* intrinsic uptake and biliary clearance that we previously measured for adults.

Because protein binding is known to affect drug clearance, especially of those drugs that possess high protein binding like micafungin, we measured $f_u$ of micafungin *in vitro* in serum from neonates and adults; as mentioned in the results section, we observed a significantly higher unbound fraction in neonates. McNamara and Alcorn [12] have shown that for lipophilic drugs, ~ 2-fold higher $f_u$ values can be expected in pediatric plasma compared to adults based on lower plasma protein concentrations in neonates than in adults. However, the model that was developed by McNamara and Alcorn [12] under-predicted the $f_u$ value of micafungin in neonates compared to the value we have experimentally measured in the present study using *in vitro* equilibrium dialysis in neonate serum. One explanation for the 8-fold higher $f_u$ in neonates compared to adult could be that micafungin binds not only to plasma proteins [more likely to albumin than gamma-globulin (31)], but also to lipoproteins, cholesterol, or other serum components that were found to be higher in adult serum than in neonate serum [12,15]. Furthermore, the physicochemical properties of a peptide like micafungin differ substantially from those of the model drugs used to establish the model obtained by McNamara and Alcorn [12]. Due to limited availability of clinical pharmacokinetic data in neonates, it is presently unclear to what extent lower protein binding (higher free fraction in the blood) in neonates versus adults is a more common reason for enhanced drug clearance in neonates. In addition, in contrast to what we have presently observed for micafungin, the enzyme or transporter-mediated elimination pathways of many drugs show pronounced age-dependency [32–34], thus complicating the interpretation of possible effects of altered protein binding on overall *in vivo* drug elimination. Caspofungin, which is another echinocandin antimycotic, also shows a small unbound fraction in blood (about 3.5%) [35]. However, in contrast to micafungin, caspofungin clearance in neonates (< 3 months) is significantly lower as compared to older children (2-11y) and, to a lesser extent, as compared to adults [36]. For this reason, the caspofungin recommended dose (normalized for body surface area) in neonates is about half the dose used in adults. While the caspofungin free fraction in neonate serum has not been measured, there is clearly no evidence that reduced caspofungin serum binding in neonates plays an important role in altered caspofungin disposition in this age group. The immunosuppressant cyclosporin A is another cyclic peptide exhibiting low unbound fraction in blood as well as age-dependent pharmacokinetics, with 2-fold lower oral clearance in neonates compared to older children and adults [37]. However, cyclosporin A shows a complex binding profile in plasma (mainly to lipoproteins) as well as in blood (distribution to erythrocytes) [38], while the ontogeny of this behavior has not been investigated. From a more general point of view, the clinical relevance of plasma protein binding alterations (most often considered from a binding competition perspective) as a mechanism underlying drug clearance differences should not be overestimated [39–40]. Nevertheless, the 8-fold increase in unbound micafungin in neonates as compared to adults is substantial, especially in light of the less pronounced effects reported previously for a list of more than 25 drugs [12]. In this list, the largest differences were reported for propranolol, quinidine and verapamil, which all exhibited 3–4 fold increased unbound fractions in neonates compared to adults. Unfortunately, clinical PK data in children, especially neonates, are very limited or lacking. In the case of verapamil, Piovan et al. [41] reported plasma levels of verapamil and its metabolite norverapamil in children between 15 days and 17 years. Their data demonstrated gradually increasing norverapamil/verapamil levels from birth onwards to reach a plateau by the age of 1 year, which then remained constant until adulthood. There was no evidence for any effect of an increased free verapamil fraction on verapamil clearance in neonates as compared to older children. This further suggests that for many drugs, altered plasma protein binding in neonates does not have a substantial impact on

clearance. In addition, even when some effect would exist, it may not be easily observed due to concomitant changes in activity of drug metabolizing enzymes and transporters [32–34].

As we have indicated in the results section (Table 1), the *in vivo* plasma clearance and biliary clearance values we calculated for micafungin in neonates were found to be three times those in adults, thus mirroring the difference in clearance between neonates and adults observed in the clinic. Since the calculated *in vivo* clearance was based on the adult intrinsic clearance values, the higher $f_u$ value of micafungin in neonates, is the factor that explains the higher *in vivo* clearance of micafungin in neonates. According to the well-stirred model applied here, neither the different relative liver protein content (mg/kg body weight) nor the higher $Q_H$ in neonates, contributed to the higher *in vivo* clearance of micafungin in neonates.

To summarize, similar protein expression of liver drug transporters, but higher $f_u$ of micafungin in neonates compared to adults have been determined by using *in vitro* methods and biological samples from both age groups. The *in vivo* plasma and biliary clearance in neonates calculated based on *in vitro* transport data of adults generated by SCHH indicated that $f_u$ may be the key contributor to the higher clearance of micafungin observed in neonates, however a definitive conclusion could not be made with the results that have been presented in this study. For instance, a possible role of one or more transporters that differ from those identified in adults may still be involved in micafungin disposition in neonatal liver. In addition, we assumed that neonates display no additional enzymatic clearance mechanisms for micafungin as compared to adults. However, Hope *et al*. [7] recently reported that substantially higher levels of the metabolite M5 were measured in neonates as compared to adults. It is clear that the existence of a neonate-specific metabolic pathway for micafungin could also contribute to its higher clearance, but further research is required in this respect. The extent of renal excretion of micafungin (and/or) metabolites in neonates has not been measured. However, based on mass balance data obtained in adults, and based on the fact that no dose adjustments are needed in patients with renal impairment [7], renal micafungin elimination was demonstrated to play a very minor role as opposed to fecal elimination. This suggests that the several fold higher clearance in neonates compared to adults is unlikely to be due to (much) enhanced renal clearance in neonates compared to adults. Future non-clinical work in sandwich-cultured hepatocytes from juvenile animals or using apical and basolateral membrane vesicles prepared from human neonatal frozen liver should be conducted in order to ultimately understand all mechanisms underlying altered micafungin disposition in neonates.

## Acknowledgments

**Financial support**: Financial support by NICHD (NCC-PPRU 5U10 HD045962-04), FWO Vlaanderen and Onderzoeksraad K.U. Leuven is acknowledged. Dr. Benjamin receives support from the United States Government for his work in pediatric and neonatal clinical pharmacology (1R01HD057956-02, 1R01FD003519-01, 1U10-HD45962-06, 1K24HD058735-01, and Government Contract HHSN267200700051C), the nonprofit organization Thrasher Research Foundation for his work in neonatal candidiasis (http://www.thrasherresearch.org), and from industry for neonatal and pediatric drug development (http://www.dcri.duke.edu/research/coi.jsp).

## Abbreviations

| | |
|---|---|
| **BCRP** | Breast Cancer Resistance Protein |
| **BSEP** | Bile Salt Export Pump |
| **Cl** | Clearance |
| **$f_u$** | fraction of drug unbound |
| **HPLC** | High Performance Liquid Chromatography |

| | |
|---|---|
| **MRP2 and MRP3** | Multidrug Resistance-associated Protein 2 and 3 |
| **NTCP** | $Na^+$-Taurocholate Co-transporting Peptide |
| **OATP** | Organic Anion Transporting Polypeptide |
| **P-gp** | P-glycoprotein |
| **$Q_H$** | hepatic blood flow |
| **SCHH** | Sandwich-Cultured Human Hepatocytes |

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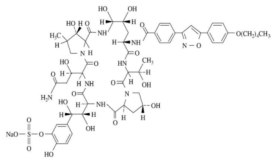

**Figure 1.**
Micafungin chemical structure

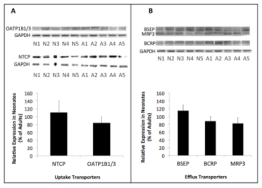


**Figure 2.**

Expression of Transporter Proteins in Liver from Human Neonates and Adults. Western blot analysis on liver homogenates from neonates and adults was used to determine the relative expression of hepatic transporters involved in micafungin uptake and biliary excretion. The top parts of panel A and panel B depict the protein expression bands in five neonate samples (N1–N5) and in five adult samples (A1–A5) for uptake and efflux transport proteins, respectively. GAPDH protein expression was measured for normalization. The bottom parts of both panels represent the mean (± SD) of GAPDH-normalized expression of the transporters in neonates (per kg body weight) relative to protein expression (per kg body weight) in adults where adult expression is 100%. Samples N3 and A5 were excluded from densitometric analysis for NTCP due to irregular band shape.

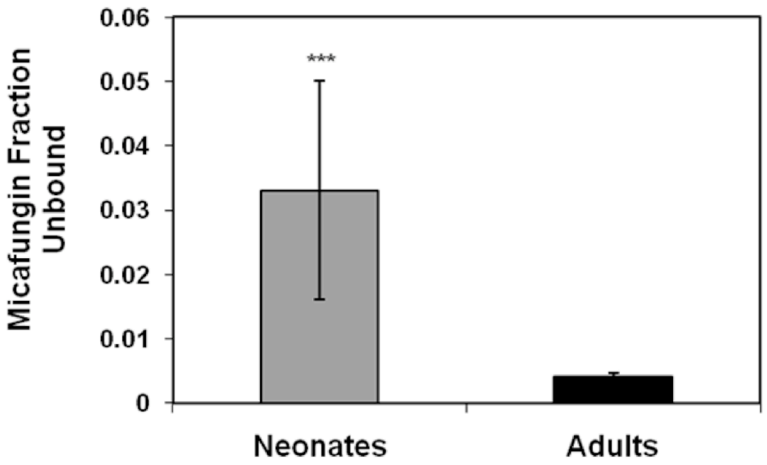


**Figure 3.**

Micafungin Fraction Unbound in Serum from Human Neonates Compared to Adults. Fraction unbound in serum from neonates and adults was determined by equilibrium dialysis as discussed in the methods section. Bars represent the mean (±SD) of values from 6 subjects/group. Statistical significance indicated by ***, P <0.001 based on the unpaired *t*-test (unequal variance).

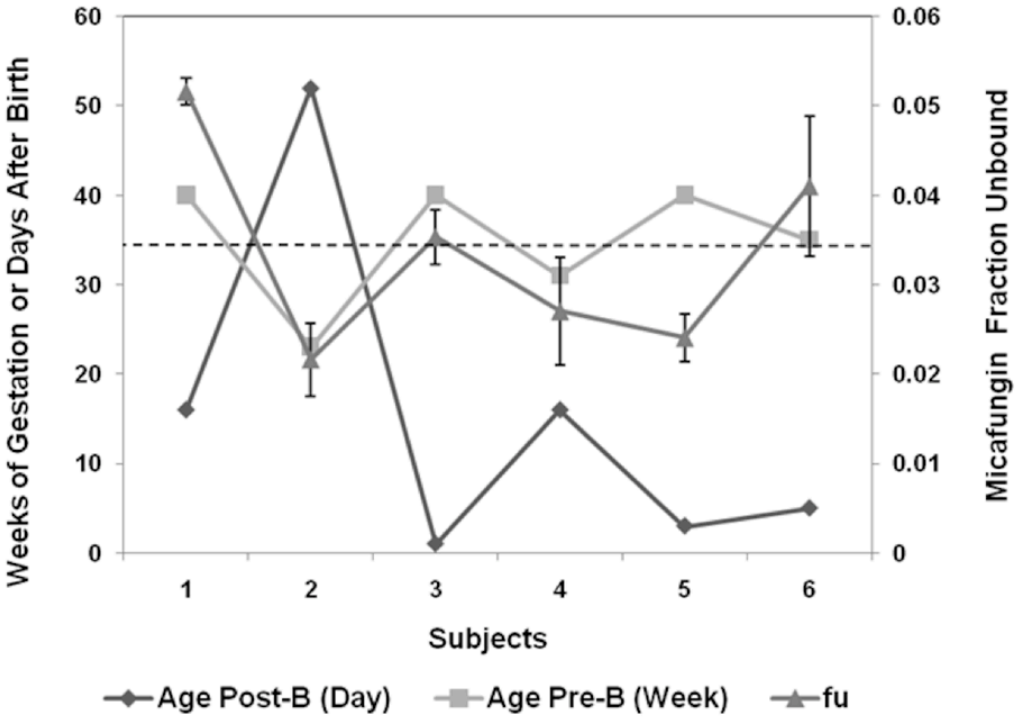


**Figure 4.**

Micafungin Fraction Unbound in Serum from Neonates. Grey triangles (▲) represent the $f_u$ (mean ± SD for three determinations) of micafungin in each subject. Each subject's postnatal age (◆) or postgestational age at birth (■) are also plotted. The mean value of $f_u$ for the six subjects is represented by a dashed line (--).

## Table 1

Calculation of *in vivo* Plasma and Biliary Clearance of Micafungin in Adults and Neonates

| Physiological Parameters & Scaling Factors | Adult* | Neonate |
|---|---|---|
| $f_u$ | 0.004 | 0.033 |
| mg protein/g liver | 71 | 24** |
| g liver/kg body weight | 26 | 43*** |
| QH, ml/min/kg | 21 | 73*** |
| Observed Plasma Cl (ml/min/kg) | **0.229** | **0.622**# |
| Intrinsic Accumulation Cl (ml/min/mg) | 0.024 ± 0.002 | 0.024 ± 0.002 |
| Intrinsic Plasma Cl (ml/min/kg) | 43.4 ± 3.0 | 24.3 ± 2.6 |
| **Calculated Plasma Cl** (ml/min/kg) | **0.175** ± **0.012** | **0.793** ± **0.085** |
| Observed Biliary Cl (ml/min/kg) | **0.160** | **Unknown** |
| Intrinsic Biliary Cl (ml/min/mg) | 0.014 ± 0.009 | 0.014 ± 0.012 |
| Intrinsic Biliary Cl (ml/min/kg) | 25.5 ± 15.7 | 14.3 ± 8.8 |
| **Calculated Biliary Cl** (ml/min/kg) | **0.101 ± 0.062** | **0.467** ± **0.287** |

Intrinsic clearance values were generated as described in the methods section. Intrinsic biliary clearance was determined using B-CLEAR® technology Qualyst, Inc.).

* Yanni *et al*. [8];

** Barter *et al*. [18];

*** Björkman [16];

# Heresi *et al*. [5].