Physiologically-Based Pharmacokinetic (PBPK) Modeling of Fluconazole Using Plasma and Cerebrospinal Fluid Samples from Preterm and Term Infants

SUPPLEMENTARY MATERIAL S1 – Supplementary Information

QUANTITATION OF CLINICAL SAMPLES

For both studies, a validated high performance liquid chromatography/tandem mass spectrometry method was used for quantifying fluconazole concentrations. The prophylaxis study was analyzed via a method developed by a central laboratory (OpAns, LLC, Durham, NC, USA), and the Pediatric Pharmacology Research Unit (PPRU) study's analytical method description has been previously published.[1] For the prophylaxis study, samples were extracted from both matrices by a 1:5 ratio addition of sample with an internal standard (IS) extraction solution (IS in acetonitrile with 2% methanol and 0.5% formic acid), followed by centrifugation and addition of water containing 0.2% (v/v) acetic acid. Samples were injected onto the instrument using mobile phases of water and acetonitrile, both with 0.2% (v/v) acetic acid. In both assays, fluconazole was measured as the product ion transition of m/z 307.1 to 220 using fluconazole-d4 as an IS.[1] Samples were quantitated using a series of standards prepared in plasma and cerebrospinal fluid (CSF) ranging 10-10,000 ng/mL with a curve fitted through a weighted $1/x^2$ regression.[1] All standards were within 15% of the theoretical value with the exception of the lower limit of quantitation, which was within 20%, and accuracy and precision values did not exceed 15% of theoretical values as per the bioanalytical method validation guidelines set forth by the U.S. Food and Drug Administration.[1] Scavenged sample concentrations were previously found to be stable and indistinguishable from timed samples.[2]

EVALUATION OF ADULT PBPK MODEL WITH CSF DATA

To establish confidence in the previously developed adult physiologically-based pharmacokinetic (PBPK) model, CSF exposure simulations were evaluated using adult data.[3] Fifty-two observed CSF samples reported in the literature from 43 adults with cryptococcal meningitis receiving fluconazole, the majority of whom received 800 mg daily oral doses, were digitized using Graph

Grabber© (Version 2.0, Quintessa, quintessa.org) and used for adult model evaluation.[3,4] Since oral dosing was not included in the previously developed PBPK model, intestinal transcellular permeability was optimized to 0.08 cm/min, and oral dosing was incorporated with time to 50% dissolution of 240 min, no lag time, and a 0.92 dissolution shaping factor.[4] Despite not being originally developed or evaluated with oral data, the previously developed PBPK model was successfully able to capture 64% of these observed data within the 90% model prediction interval (**Figure S2**). Further, the model predicted CSF-to-plasma ratio was 0.84, which is in line with previous findings of 0.89 in adults.[5,6] This provides further confidence in the scaled pediatric model's ability to predict fluconazole's distribution in the central nervous system.

PBPK MODEL SCALING

Clearance was scaled from adults to infants by scaling both glomerular filtration rate (GFR) (renal clearance) and 5'-diphosphoglucuronosyltransferase 2B7 (UGT2B7) (hepatic clearance) assuming 85% renal clearance and 15% hepatic clearance solely by UGT2B7.[4,7,8] Scaling accounts for both gestational age (GA) and postnatal age (PNA) by using the composite postmenstrual age (PMA) measure in sigmoidal equations for the GFR and UGT2B7 ontogeny functions. Size-normalized clearance was scaled linearly by dividing the clearance value by individual weight.

Renal clearance was scaled to infants as the percentage of adult GFR corrected by fraction unbound according to Equation 2:

$$CL_{GFR,child} = \frac{GFR_{child}}{GFR_{adult}} * \frac{f_{u,child}}{f_{u,adult}} * CL_{GFR,adult} \qquad (1)$$

where $CL_{GFR,child}$ is the infant's clearance as a function of GFR, $GFR_{(child)}$ is the estimated infant GFR, GFR_{adult} is the adult GFR (110 mL/min), $f_{u,child}$ is the fraction unbound in infants, $f_{u,adult}$

is the fraction unbound in adults, and $CL_{GFR(adult)}$ is the adult clearance as a function of GFR.[4] GFR_{child} was estimated using a sigmoidal PMA model established by Rhodin *et al*[9]:

$$GFR_{child} = GFR_{adult} * \left(\frac{0.74*PMA^{15.0}}{(44.4^{15.0}+PMA^{15.0})} + 0.26\right) \quad (2)$$

which also incorporates a small fractional offset of 0.26 to correct for the sigmoidal PMA model's tendency to slightly underestimate the GFR for preterm infants below 32 weeks GA.[10]

Hepatic clearance via UGT2B7 was also scaled as a function of PMA according to the following equation[11]:

$$OSF_{UGT2B7} = \frac{PMA^{6.543}}{(72.533^{6.543}+PMA^{6.543})} \quad (3)$$

where OSF_{UGT2B7} is the ontogeny scaling factor for UGT2B7. Hepatic clearance was scaled to infants by adjusting adult unbound hepatic intrinsic clearance by age- and enzyme-specific percent of adult activity (ontogeny) using Equation 4:

$$CL_I{}'_{UGT2B7(child\ g\ liver)} = OSF_{UGT2B7} * CL_I{}'_{UGT2B7(adult\ g\ liver)} \quad (4)$$

where $CL_I{}'_{UGT2B7(child\ g\ liver)}$ is the scaled unbound hepatic intrinsic clearance due to UGT2B7 per gram of liver and $CL_I{}'_{UGT2B7(adult\ g\ liver)}$ is the adult unbound hepatic intrinsic clearance due to UGT2B7.[4]

Binding to alpha-1-acid glycoprotein was scaled using a recently developed ontogeny sigmoidal equation as a function of PNA only[12]:

$$fu_{child} = \left(\frac{1}{\left(1+\frac{PNA^{0.735}}{(11.53^{0.735}+PNA^{0.735})}\right)} * \frac{(1-fu_{adult})}{fu_{adult}}\right) \quad (5)$$

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