In Vitro Techniques to Predict Intestinal First-pass Metabolism in Children and Adults
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Introduction

Voriconazole is a broad spectrum triazole antifungal agent used for life-threatening fungal infections, especially in immunocompromised patients. It is cleared predominantly by oxidative metabolism by cytochrome (CYP) 3A4, CYP2C19, and flavin-containing monoxygenase (FMO) 3 to an N-oxide and an alcohol (Figure 1).

Oral bioavailability of voriconazole is approximately half in children (age 2-10 years) compared to adults. Physiologically based pharmacokinetic models developed and reported by the Thakker laboratory suggest that voriconazole undergoes intestinal first-pass metabolism in children, but not in adults.

Intestinal first-pass metabolism of an oral drug can clear a large portion of the dose before it reaches the systemic circulation (Figure 2). A compartment “intestinal cytochrome P450 pie” for adults is depicted in Figure 3. However, this information is not available for children. Current techniques used to study intestinal metabolism in children rely on scaling adult data.

The aim of this study was to develop a method to study differences in intestinal metabolism between adults and children.

Materials and Methods

Sample Selection: Post-mortem intestinal tissues from healthy adults (>18 years old) and children (aged 2-10 years) were obtained from the NICHD Brain and Tissue Bank Contract #HHSN20009000011C, Ref. No. NO1-HD-9-0011, Baltimore, MD, under an approved UNC-Chapel Hill IRB. Discarded surgical jejunal samples were procured from patients undergoing gastric bypass at UNC Hospitals.

Gene Expression: mRNA was isolated from tissues (25mg) using a Qiagen RNaseasy Kit, and mixed with (Script™) cDNA Synthesis Kit (Biorad) to synthesize cDNA. The cDNA was mixed with Taq™ Supermix (BioRad) and TagMan Gene Expression Assay mix for CYP2C9, 2C19, and 18s (Applied Bio-systems). Expression for each enzyme was measured by quantitative RT-PCR and normalized to 18s RNA.

Enterocyte Isolation: Intestinal samples were incubated in Buffer 1 (1.5mM KC, 96mM NaCl, 27mM sodium citrate dehydrate, 8mM KH2PO4, 5.65mM NaH2PO4) for 30 min. The solution was replaced by Buffer 2 (1.5mM EDTA, 3 units/mL heparin, and 0.5mM dithiothreitol), tissue was shaken for 15 min, and the procedure repeated 6-7 times. After centrifugation, the pellet was used to create human intestinal microsomes (HIMs).

Microsome Preparation: Microsomes were prepared as previously reported. Microsomal protein concentration was measured using a BCA assay.

Functional assay: Adult and pediatric HIMs were incubated with midazolam or voriconazole (4 μM, respectively), NADPH (1 mM) and McgCl2 (3 mM) in phosphate buffer (pH 7.4). Aliquots were taken at specified times, quenched with 300 μL ice-cold methanol containing the internal standard (alprazolam or diclofenac), centrifuged and analyzed by LC/MS/MS.

Metabolite Analysis: The LC/MS/MS system consisted of mobile phase (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile with a total flow rate of 0.7 mL/min. Samples were ionized using an APCI ion source and following injection of supernatant, positive ions were monitored.

Conclusions and Discussion

1. Intestinal tissue samples begin to rapidly degrade within 2 hours post-mortem.
2. Conversely, surgical intestinal tissue samples retain greater metabolic activity, even when stored at -80°C prior to preparation and functional analysis.
3. Only a small sample of intestinal tissue is needed (~2 inches) when using a combination of elution and homogenization; thus this method should be used to study intestinal metabolism in children.
4. If intestinal samples from pediatric surgical patients are obtained, intestinal first-pass metabolism can be predicted in children (and adults) by integrating in vitro metabolism data into a physiologically based pharmacokinetic model.

This information is valuable in the screening and optimization of new drug candidates for adult and pediatric indications.

References


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