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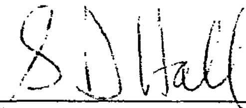
INFLUENCE OF DIFFERENT CYP3A ENZYMES, PROBE SUBSTRATES, AND KINETIC MODELS ON PREDICTIONS OF IN VIVO DRUG-DRUG INTERACTION POTENTIAL

Jessica L. Fayer

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Master of Science
in the Department of Pharmacology and Toxicology
Indiana University

August 2005

Accepted by the Faculty of Indiana University, in partial fulfillment of the requirements for the degree of Master of Science.



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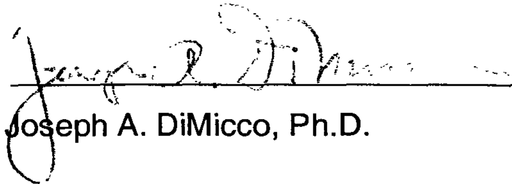

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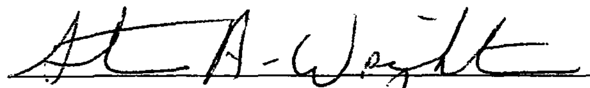

Steven A. Wrighton, Ph.D.

Jessica L. Fayer

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CYP3A4 is important in drug metabolism and drug-drug interactions (DDI), but the importance of the highly-related CYP3A5 is debated. CYP3A enzymes may exhibit unusual enzyme kinetics, including substrate-dependent inhibition and other non-Michaelis-Menten kinetics. Therefore, to determine the kinetics and inhibition (n = 5 inhibitors) of the two enzymes, two distinct CYP3A-specific marker substrate activities (midazolam 1'-hydroxylation and testosterone 6β-hydroxylation) were employed. CYP3A4 and CYP3A5 activities, indistinguishable in hepatic microsomal preparations, were examined independently with the use of cDNA-expressed enzymes. The simple Michaelis-Menten model was a good fit for midazolam 1'-hydroxylation data for both CYP3A4 and CYP3A5. Unusual kinetics were observed for testosterone 6β-hydroxylation, so the better of the fits of the Michaelis-Menten and the Hill model for autoactivation was determined for CYP3A4 (Michaelis-Menten) and CYP3A5 (Hill model). Efficiencies for the marker substrate reactions catalyzed by CYP3A4 and CYP3A5 were similar, indicating that CYP3A5 may be an important contributor to metabolism in individuals who express substantial levels of

CYP3A5 relative to CYP3A4. Inhibitory potency (K_i) as measured by midazolam 1'-hydroxylation was determined with a simple competitive inhibition model. For testosterone 6β-hydroxylation, CYP3A4 displayed relatively little sigmoidicity. Thus, the competitive inhibition model was judged to be appropriate for the inhibition of testosterone 6β-hydroxylation by the five inhibitors. However, CYP3A5 displayed marked sigmoidicity, and a more complex 3-site model was necessary to determine the potential for inhibition of CYP3A5-mediated testosterone 6β-hydroxylation. Potency of the inhibitors was consistently much greater for CYP3A4 than CYP3A5. There was no clear pattern of inhibitory potency associated with choice of marker substrate reaction. Finally, in vitro K_i values were related to in vivo circulating drug concentrations to gauge the potential for clinically-significant DDI. Only the inhibitor cyclosporine was predicted to cause DDI mediated via CYP3A4, based on the clinically-achievable concentrations exceeding in vitro K_i values. Lack of inhibition of CYP3A5 by the inhibitors in this study indicates that individuals expressing high levels of CYP3A5 relative to CYP3A4 may be less susceptible to DDI than those expressing minimal or no CYP3A5. CYP3A5 should be considered when identifying the enzymes responsible for drug metabolism and the potential for DDI.

Stephen D. Hall, Ph.D., Chair

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