Short Communication

Human Cytochrome P450 Maximal Activities In Pediatric versus Adult Liver

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ABSTRACT:

Drug clearance is often higher in children than in adults, particularly when normalized to body weight. We previously showed that liver volume normalized to body weight was inversely related to age, but that the systemic clearance of a nonspecific cytochrome P450 substrate (antipyrine) was higher in young children compared with adults even when normalized per liver volume. Our purpose herein was to evaluate whether P450 catalytic activities, expressed as maximal catalytic rates per milligram of microsomal protein, differed in up to 37 normal livers from subjects <10 (range 0.5–9 years of age), >10 but ≤60 years of age (range 10–59 years), and >60 year (range 63–93 years of age). There were no age-related differences in the oxidation of ethoxyresorufin (P = .83) for multiple comparisons. No relationship was found to exist between age and microsomal recovery (P = .98); thus, recovery did not account for the lack of age-related differences in catalytic activity. We conclude that increased intrinsic cytochrome P450 activity is unlikely to account for increased clearance of most P450 drug substrates in children.

There are many drugs that exhibit a higher systemic clearance in children than in adults, especially when normalized to body weight (Evans et al., 1989). Several of these drugs, including theophylline (Ellis et al., 1976), antipyrine (Crom et al., 1991), teniposide (Evans et al., 1982), phenytoin (Curless et al., 1976), and quinidine (Szefler et al., 1982) are eliminated primarily through hepatic metabolism. Antipyrine, which is metabolized by cytochrome P450 3A4, is expressed as maximal catalytic rates per milligram of microsomal protein, differed in up to 37 normal livers from subjects <10 (range 0.5–9 years of age), >10 but ≤60 years of age (range 10–59 years), and >60 year (range 63–93 years of age). There were no age-related differences in the oxidation of ethoxyresorufin (P = .83) for multiple comparisons. No relationship was found to exist between age and microsomal recovery (P = .98); thus, recovery did not account for the lack of age-related differences in catalytic activity. We conclude that increased intrinsic cytochrome P450 activity is unlikely to account for increased clearance of most P450 drug substrates in children.

Materials and Methods

Human Tissues. Human livers were obtained from the Liver Tissue Procurement and Distribution System (Minneapolis, MN), Cooperative Human Tissue Network (Birmingham, AL), Dr. Urs Meyer (University of Basel), and Dr. Erin Schuetz (St. Jude Children’s Research Hospital, Memphis, TN). Only livers that were histologically normal at the time of procurement and obtained through biopsy or within 1 h of “cross-clamp” (in the case of organ donors) were used in the analyses of P450 catalytic activities. For the majority of livers, complete drug history before liver collection was not available. Because catalytic activities were not found to differ between livers from individuals who were known to have received potent P450 inducers (e.g., glucocorticoids, phenytoin, and barbiturates) and those who were not, “induced” livers were included in all other comparisons. Microsomes were prepared and protein was measured as reported previously (Relling et al., 1992). Microsomal recovery (expressed as a percentage, %) was estimated using the following formula: total amount of microsomal protein/wet weight of frozen liver × 100.

P450 Catalytic Activities. The substrates used in this study were teniposide (CYP3A4/5 substrate), ethoxycoumarin (CYP2E1 and other P450s substrate), midazolam (CYP3A4 and CY3A5 substrate), paclitaxel (CYP2C8), tolbutamide (CYP2C9), and ethoxyresorufin (CYP1A2 substrate). Maximal P450 catalytic activities were assessed by incubating 0.1 to 0.3 mg of microsomal protein in duplicate with prototypical substrates for specific P450s; not all activities were measured in all livers. Details for teniposide (Relling et al., 1994), midazolam (Relling et al., 1994), paclitaxel (Sonnichsen et al., 1995), ethoxycoumarin (Evans and Relling, 1992), and tolbutamide (Relling et al., 1990) incubations and assays were reported previously. “Maximal” catalytic activities at 500-, 60-, 20-, 1000-, and 2000-μM substrate concentrations, respectively, were assessed. Ethoxyresorufin (5 μM) deethylation was assessed using a modification of a fluorimeter-based method (Lubet et al., 1985). Briefly, 0.2 mg of microsomal protein, 10 μl of NADPH regenerating system, in a total volume of 100 μl of 0.1 M TRIS-HCl buffer (pH 7.8) were incubated at 37°C for 5 min. The reaction was stopped by addition of 100 μl of cold methanol. After low-speed centrifugation, 50 μl were injected onto an HPLC system.
using a Bondapak Phenyl column (Waters, Milford, MA) with conditions essentially as described (Evans and Relling, 1992). Resorufin was quantitated using calibrators prepared exactly as described for unknowns but having resorufin spiked after the addition of methanol to microsomes and NADPH. All catalytic activities are expressed as nanomoles of metabolite formed per hour per milligram of microsomal protein.

Statistical Methods. Wilcoxon rank-sum tests were used to determine whether or not the distributions of two groups of maximal catalytic activity assay results differed, whereas Kruskal-Wallis H tests were used to determine whether or not at least one of the distributions of $k > 2$ groups of catalytic activity assay results differed. Exact methods were used when necessary. Spearman’s rank correlation coefficient was used to determine whether or not a relationship existed between age or maximal activities and microsomal recovery.

No adjustments have been made to the $P$ values included in this report. However, Bonferroni adjustments were made to the significance levels to control for multiple testing within each catalytic activity. The overall significance level within each activity was controlled at $\alpha = 0.05$.

All analyses were conducted using SAS Release 6.12 and StatXact-3 Version 3.0.2.

**Results**

Maximal catalytic activity assays were conducted in 52 normal livers; not all activities were assayed in each liver sample. The age range for the youngest group ($<10$ years, $n = 3–13$) was 0.5 to 9 years of age, for the intermediate group ($>10$ to $<60$ years, $n = 7–19$) was 10 to 59 years of age, and for the oldest group ($>60$ years) was 63 to 93 years of age. As most studies show little difference in clearance among children over 10 years of age and adults (Ellis et al., 1976; Crom et al., 1991), only those $<10$ years of age are denoted “children” in the following analyses.

Figure 1 shows the distributions of each cytochrome P450 catalytic activity in the three defined age groups. There were no differences between the three age groups in the oxidation of the following:

**Fig. 1.** Box plots depicting median, interquartile range, and absolute range of catalytic activities (nmol/mg/h) by age group for ethoxyresorufin deethylation (CYP1A2), teniposide O-demethylation (CYP1A4/5), ethoxycoumarin deethylation (CYP2E1 and others), midazolam 1$^1$-hydroxylation (CYP3A4/3A5), paclitaxel 17a hydroxylation (CYP2C8), and tolbutamide hydroxylation (CYP2C9).
These enzymes constitute over 80% of the P450 enzymes present in CYP3A4/3A5, CYP2C8, and CYP2C9 from the microsomal fraction (Murry et al., 1995). To test this hypothesis, we evaluated the catalytic activities observed in younger children compared with adolescents (Murry et al., 1995). These data fail to indicate a trend for age-related differences in maximal P450 catalytic activity and P450 oxidation rates.

Discussion

In previous clinical pharmacokinetic studies, we proposed that greater overall catalytic activity of hepatic drug-metabolizing enzymes could have contributed to the high antipyrine clearance that we observed in neonatal children. A lack of relationship between age and hepatic microsomal P450 activity (Shimada et al., 1994; CYP3A5 activities are comparable in children and adults (Vieira et al., 1996; Lacroix et al., 1997). Taken together with our findings, there are not data to support the hypothesis that P450 activities are higher in the 1- to 10-year age range compared with other ages. Because it is this group that tends to exhibit higher drug clearance clinically, it appears that other mechanism(s) must account for their higher clearance. Our findings that in vitro maximal catalytic activities do not mirror the increased drug clearance of children are consistent with and complementary to previous studies showing comparable maximal catalytic activities in adult versus elderly livers (Brodie et al., 1981).

There are several factors other than maximal P450 activity that could contribute to age-related changes in drug clearance, in vivo, and could account for the lack of correspondence between in vitro and clinical findings. Substantial reductions in blood flow and hepatic size contribute to reduced clearance in the elderly (Dawling and Crome, 1989). Many high intrinsic-clearance-drugs that are cytochrome P450 substrates, for example, erythromycin (Miglioli et al., 1990), nifedipine (Robertson et al., 1988), and hexobarbital (Chandler et al., 1988) have lower elimination in the elderly than in young adults (Kinirons and Crome, 1997). However, for P450 substrates that are low intrinsic-clearance-drugs, clearance is not significantly dependent on hepatic blood flow, and, thus, higher hepatic blood flow is unlikely to account for most higher P450 drug substrate clearances in children.

Another explanation that has been put forward to explain lower drug clearance in the elderly is the “oxygen limitation” theory (Le Couteur and McLean, 1998). According to this hypothesis, the hepatic oxygen supply for phase I enzymatic reactions appears to be compromised among the elderly. This effect may not have been observed in our microsomal model because oxygen supply is not as constrained in vitro as it might be in vivo. It is conceivable that transcellular uptake of oxygen, substrates, or cofactors that facilitate metabolism are more efficient in pediatric liver, and these uptake effects would not be observable with in vitro microsomal studies. Factors that affect enzyme-substrate affinity or endoplasmic reticulum availability of either substrate or enzyme could vary as a consequence of subtle age-related changes in the hepatocyte structure or enzyme conformation (e.g., glycosylation status, folding, etc.), and these factors would not have been appreciated in our analysis of maximal catalytic activity in microsomes. Moreover, if microsomal recovery had been reduced in pediatric relative to adult liver, maximal activities normalized to microsomal protein concentration would have been low relative to that normalized to total pediatric liver. However, we found no relationship between age and microsomal recovery, nor any relationship (nor was any expected) between recovery and maximal catalytic activity.

We conclude that increased maximal P450 activity, as a function of amount of hepatic microsomal protein, is unlikely to account for the
higher clearance observed for most P450 drug substrates in children versus adults.

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References


