EFFECTS OF GENDER, AGE AND ETHNICITY ON HUMAN CYTOCHROME P450 ACTIVITY

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INTRODUCTION

It has been well documented that the expression of CYP enzymes is influenced by endogenous factors, such as genetic polymorphisms and hormone levels, and by exogenous factors, such as diet, exposure to drugs, alcohol consumption, cigarette smoking and various environmental factors (Parkinson, *et al.*, 2004). For this study, we examined the effects of three endogenous factors and two exogenous factors on individual CYP activities in over 300 samples of human liver microsomes. The aim of this analysis was to evaluate whether the age, gender, or ethnicity of the donor should influence the selection of human liver microsomes for drug metabolism studies, as well as whether cigarette smoking and alcohol consumption are reliable indicators of elevated CYP1A2 and CYP2E1 activity, respectively.

MATERIALS & METHODS

The sources of human livers (and the attendant ethical, confidentiality, and safety issues) and the procedures we use to prepare liver microsomes and measure CYP enzyme activities have been described elsewhere (Pearce, *et al.*, 1996). All of the data presented below were obtained with human livers that were initially procured for transplantation purposes. All donors were free of known infectious diseases that would have precluded transplantation (such as hepatitis and HIV), and in all cases the cause of death was known.

The donor information provided with each liver is taken at face value. Some of this information, such as the age and sex of the donor, can be considered highly reliable. Other information is incomplete or more difficult to interpret. For example, information on illicit drug use, alcohol consumption, and smoking history may be incomplete or misleading.

Information on ethnicity assumes that individuals fall into discrete groups, even if the donor's parents are ethnically distinct. This complicates an evaluation of the effects of ethnicity on CYP enzyme expression. In analyzing our data, we have not applied selection criteria when evaluating the effects of any given variable on CYP enzyme activity. For example, when evaluating the effects of gender, all of the available data were used. We did not exclude samples from donors who, for example, were reportedly taking enzyme-inducing drugs. That is because our goal was to assess whether age, gender, and ethnicity should be taken into account when selecting human liver microsomes for drug metabolism studies, rather than to discern whether any of these variables have clinically relevant influences on drug metabolism (Parkinson, *et al.*, 2004).

The marker reactions used in analysis were 7-ethoxyresorufin *O*-dealkylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), *S*-mephenytoin *N*-demethylation (CYP2B6), paclitaxel 6α-hydroxylation (CYP2C8), diclofenac 4'-hydroxylation (CYP2C9), *S*-mephenytoin 4'-hydroxylation (CYP2C19), dextromethorphan *O*-dealkylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), testosterone 6β-hydroxylation (CYP3A4/5) and lauric acid hydroxylation (CYP4A11).

TABLE 1

Summary of individual demographics in the population examined

Donor Demographics			
Age Groups		**Ethnic Groups**	
0-5 years	n = 12	Caucasian	n = 239
6-20 years	n = 25	Hispanic	n = 27
21-40 years	n = 59	African American	n = 29
41-60 years	n = 144	Asian	n = 5
Over 60 years	n = 61	Not Determined	n = 1
Total	n = 301	**Total**	n = 301

Gender		**Tobacco Use**		**Alcohol Use**	
Male	n = 173	Smokers	n = 78	Drinkers	n = 136
Female	n = 128	Non-smokers	n = 218	Non-drinkers	n = 160
		Unknown	n = 9	Unknown	n = 5
Total	n = 301	**Total**	n = 301	**Total**	n = 301

Figure 1 **Effects of gender on CYP activity in human liver microsomes. No statistically significant differences were determined by linear regression analysis with the exception of CYP3A4/5, p<0.001**

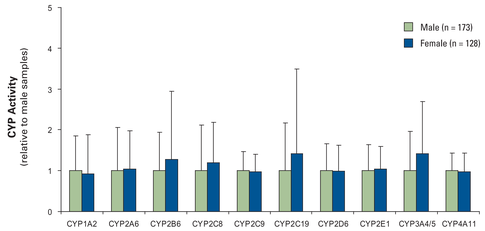


Figure 2 **Effects of age on CYP activity in human liver microsomes. No statistically significant differences were determined by linear regression analysis**

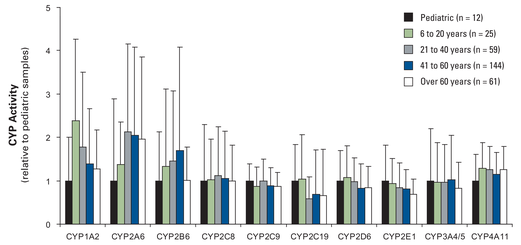
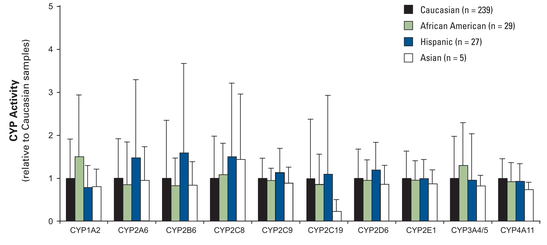


Figure 3 **Effects of ethnicity on CYP activity in human liver microsomes. No statistically significant differences were determined by linear regression analysis**



RESULTS

The demographics for the individuals used in this analysis are listed in **Table 1**. **Figures 1** through **5** show the effects of each factor on the examined CYP activities.

From the size of the error bars in **Figure 1**, it is apparent that, without exception, CYP activity varies widely in liver microsomes from both male and female donors. The differences in means between males and females are slight and not statistically significant with one exception (CYP3A4/5, p< 0.001). Despite a lack of statistical significance, there are some interesting trends. For example, CYP2B6 and CYP2C19 activity tend to be higher in liver microsomes from female donors, whereas CYP1A2 activity tends to be higher in liver microsomes from male donors. This supports gender related differences found in clinical research (Tanaka, 1999; Rasmussen, *et al.*, 2002).

The effects of age on CYP enzyme activity in human liver microsomes are shown in **Figure 2**. The subjects were segregated into five age groups: at least 12 samples in each of these five age groups. From the size of the error bars in **Figure 2**, it is apparent that, without exception, CYP activity varies widely in liver microsomes from donors of all ages, and there are no statistically significant differences in CYP enzymes among the five age groups (0 – 5 years, 6 – 20 years, 21 – 40 years, 41 – 60 years, and > 60 years old). Though not statistically significant, there was an apparent age-related decline in CYP2E1 activity.

The effects of ethnicity on CYP enzyme activity in human liver microsomes are shown in **Figure 3**. Data for Asians are available for only five microsomal samples, so these data should be interpreted with particular care. From the size of the error bars in **Figure 3**, it is apparent that, without exception, CYP activity varies widely in liver microsomes from ethnic groups. There were no statistically significant differences in CYP activity among Caucasians, African Americans, Hispanics and Asians. For CYP3A4/5, there appears to be an increase in activity for African American samples compared to the other ethnic groups. However, the difference could possibly be explained by the smaller number of African American individuals upon which the data are based. The differences seen, however, are not statistically significant. Compared with those from Caucasians, African Americans, and Asians, liver microsomes from Hispanics tended to have greater CYP2A6 and CYP2B6 activity. Liver microsomes from Hispanics and Asians had greater CYP2C8 activity, whereas African Americans tended to have greater CYP1A2 activity, and Asians had lower CYP2C19 activity when compared to the other ethnicities in this study. These apparent ethnic differences may be a consequence of the relative low number of African American, Hispanic, and Asian donors.

The two exogenous factors examined in this research, cigarette smoking and alcohol consumption, are known to cause clinically significant induction of liver microsomal CYP1A2 (Rasmussen, *et al.*, 2002) and CYP2E1 (Girre, *et al.*, 1994), respectively. **Figure 4** shows liver microsomal CYP1A2 activity as a function of gender and cigarette smoking, whereas **Figure 5** shows liver microsomal CYP2E1 activity as a function of gender and alcohol consumption. These analyses were restricted to those samples for which we received unambiguous data on each donor's smoking and drinking habits. Despite these, it is possible that the information available to us is incomplete or inaccurate. For example, some smokers may have identified themselves (or been identified by next of kin) as being non-smokers; some drinkers may have identified themselves (or been identified by next of kin) as being non-drinkers. Even when smokers and drinkers are correctly identified, it is unreasonable to assume that all these patients (some of whom may have been on life-support systems) would have continued to smoke cigarettes and consume alcohol during their hospitalization. Furthermore, our analysis does not exclude other factors, either genetic or environmental, that contribute substantially to variation in CYP1A2 and CYP2E1 levels. From a practical consideration, it might be assumed that choosing liver microsomal samples from known smokers and alcohol drinkers would be helpful in selecting those samples with high CYP1A2 and CYP2E1 activity, but the results of this study in **Figures 4** and **5**, respectively, argue against this assumption.

Figure 4 **Apparent effects of smoking cigarettes on CYP1A2 activity in human liver microsomes**

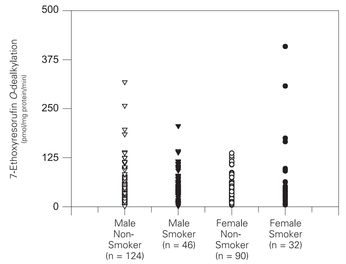
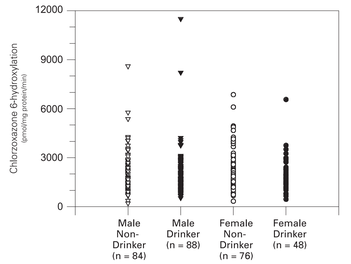


Figure 5 **Apparent effects of drinking alcohol on CYP2E1 activity in human liver microsomes**



CONCLUSION

The results of this study consistently point to the same conclusion, namely that CYP enzyme activity in human liver microsomes varies considerably from one sample to the next, and that this variation is observed in human liver samples from males and females, infants, young, middle-aged and elderly donors, and in livers from Caucasians, African Americans, Hispanics and Asians. Therefore, these variables (*i.e.,* gender, age, and ethnicity) provide no meaningful basis for selecting human-derived materials for routine studies of drug metabolism. Even variables that are known to have clinically relevant effects on CYP enzyme expression, such as cigarette smoking (which induces CYP1A2) and alcohol consumption (which induces CYP2E1) are poor predictors of CYP enzyme activity in individual samples of human liver microsomes (*i.e.,* there is a wide and largely overlapping range of CYP1A2 activity in smokers and non-smokers, and there is a wide and largely overlapping range of CYP2E1 activity in alcohol drinkers and non-drinkers).

Some differences (generally not statistically significant) in mean CYP activity were observed among groups, and some of these reflect clinical findings. For example, liver microsomal CYP1A2 activity tended to be lower in females than males, whereas CYP3A4/5 activity in microsomes tended to be higher in females than males, which is consistent with certain clinical findings (Tanaka, 1999). Also, liver microsomal samples from Hispanic donors were found to have elevated CYP2A6 and CYP2B6 activity, whereas African Americans had elevated CYP1A2 activity, and Asians had lower CYP2C19 activity. However, from the perspective of conducting routine drug metabolism studies, the magnitude of these differences is inconsequential, especially when one considers the wide inter-sample variation in CYP activities within any particular group examined.

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