

INTER-INDIVIDUAL VARIABILITY IN THE CATALYTIC ACTIVITY OF CYP3A4 PER UNIT OF ENZYME (k_{cat})

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INTRODUCTION

Inter-individual variation in *in vitro* CYP3A catalytic activity is considerable (1, 2) and is thought to be a manifestation of the high degree of variation in individual levels of hepatic CYP3A4 protein (3). Previous studies have shown significant correlations between hepatic CYP3A4 abundance and *in vitro* catalytic activity (1, 2). Typically, the rates of metabolism described in such studies are expressed in terms metabolite formation per minute per mg microsomal protein (/mmp). Our aim was to re-evaluate the variability based on the intrinsic activity of the enzyme (k_{cat}).

METHODS

Microsomes were prepared from 53 livers held within the liver bank at the Unit of Clinical Pharmacology, University of Sheffield (methods described previously) (4). Microsomal CYP3A4 abundance was quantified using a competitive Enzyme Linked Immunosorbent Assay (ELISA) (cross validated using Western Blotting: $r^2 = 0.62$, $p < 0.001$). Microsomal cytochrome b5 content was determined by dithionite difference spectroscopy as described by Klingerberg, (1958) (5). CYP3A catalytic activity was assessed by incubation of microsomal samples (0.1mg/ml protein) with 200 μ M of the CYP3A probe substrate testosterone.

RESULTS

CYP3A4 abundance ranged from 5 – 928 pmol / mg microsomal protein (197 fold) across the 53 livers.

Concentrations of 6 β hydroxy testosterone formation were below the limit of detection of the assay in 2 of the samples. Activity in the remaining 51 livers ranged from 0.3 – 11.4 nmol / min / mmp (42 fold). The distributions of activity and CYP abundance were skewed. Hence, a non-parametric approach was used to assess correlations. A significant correlation (Spearman rank = 0.58, $p < 0.01$) was found between testosterone 6 β hydroxylase activity (pmol / min / mmp) and CYP3A4 protein (pmol / mg) (Figure 1).

Correcting individual testosterone 6 β hydroxylase activities for CYP3A4 abundance to give a rate in terms of nmol / min / pmol CYP3A4 (k_{cat}) reduced variability in activity from 42 fold to 22 fold across the 51 livers.

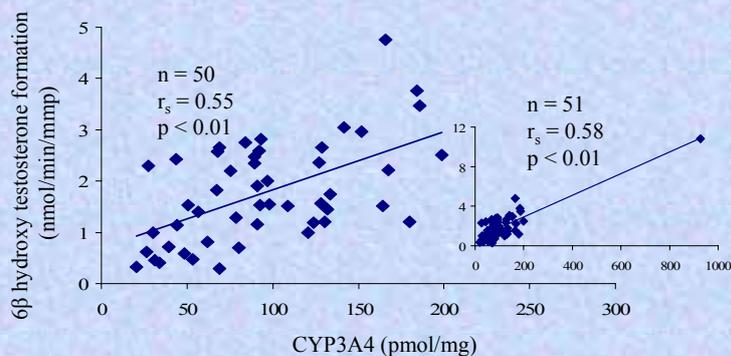


Figure 1: Spearman rank correlation of CYP3A4 protein with testosterone 6 β hydroxylase activity

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DISCUSSION

Inter-individual variation in k_{cat} was lower than that of catalytic activity (/mmp): 22 vs 42 fold, respectively.

Several livers exhibited high CYP3A activity despite having relatively low expression of CYP3A4 protein (highlighted in Figure 2a). Exclusion of these livers reduced fold variation in k_{cat} further (from 22 fold to 9 fold). As testosterone is also known to be a substrate for CYP3A5 (6), the atypically high levels of activity may be due to the presence of CYP3A5 protein. However, CYP3A5 abundance was not determined in our liver samples.

Inter-individual variability in published values (1) of catalytic activity (/mmp) was also reduced by conversion to k_{cat} (Figure 2b). Similar to our finding, Westlind-Johnsson *et al.* observed individual livers possessing low CYP3A4 expression but high CYP3A activity (Figure 2b). These samples were shown not to contain high levels of CYP3A5. It is possible that differences in levels of accessory proteins such as cytochrome b5 and NADPH cytochrome P450 reductase between individuals may be responsible for a proportion of the remaining variability in k_{cat} . However, in our study, correlation of cytochrome b5 levels with catalytic activity (/mmp) as part of a multiple regression analysis of data from 36 liver samples did not achieve statistical significance.

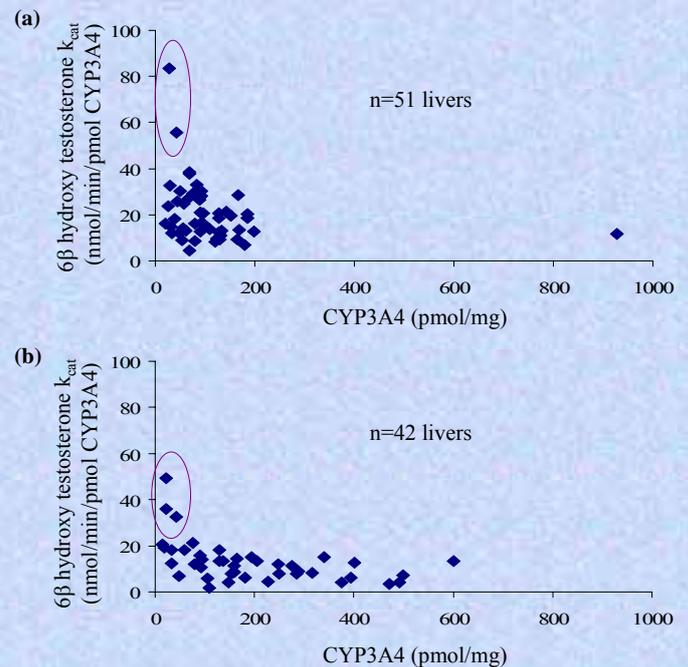


Figure 2: Relationship between CYP3A4 protein and k_{cat} (nmol/min/pmol CYP3A4) (a) in randomly selected samples from the Sheffield Human Liver Bank (b) in non randomly selected samples (Westlind-Johnsson *et al.*)

CONCLUSIONS

Inter-individual variability in CYP3A related activity is associated with variation in individual levels of hepatic CYP protein expression (determined by genetic or environmental factors). Intrinsic activity, as expressed by apparent k_{cat} is less variable. However, considerable residual variability indicates a need for future searches for covariates of activity.

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5. Klingerberg, (1958) *Arch Biochem Biophys* 75:376.
6. Kamden *et al.*, (2004) *Naunyn-Schmiedeberg's Arch Pharmacol* 370: 71