Letter to the Editor

EVALUATION OF RECOMBINANT CYTOCHROMES P450 ACTIVITY IN METABOLIC PATHWAYS

In the August issue of Drug Metabolism and Disposition, an article on the metabolism of the anticancer agent tamoxifen by Crewe et al. (2002) appeared. This article investigates the implication of cytochrome P450 (P450) isozymes involved in four different metabolic pathways.

For more than a decade, tamoxifen biotransformation was extensively studied since its 4-hydroxy metabolite showed much more potent anti-estrogen efficacy than its parent drug and because protein and DNA adducts were discovered in animals as well as in treated women (Phillips, 2001). Therefore, correlations between tamoxifen resistance or toxicity and drug metabolism were investigated but unfortunately until now without real success.

Recently, an interesting article reviewing tamoxifen resistance at the molecular level (Clarke et al., 2001) described mechanisms of resistance depending on the cell clones. In other words, one cell clone could show a resistance related to its metabolism whereas another resistant clone would not. Such heterogeneity of drug resistance underlines the need to investigate drug metabolism as a possible mechanism of resistance even if some data suggest the contrary in a specific cell clone. Thus, identifying P450s involved in tamoxifen metabolism remains a challenge in order to investigate a resistance to tamoxifen.

It is clear that the interest is to identify P450s implication in vivo in the tumor cells or in the liver cells. In Crewe et al. (2002), various cDNA-expressed P450s (recombinant systems) were used to identify the specific isozyme(s) involved in four different metabolic pathways. For such investigation, the use of recombinant P450 isozymes seems one of the best tools.

In this article, results are given at best by metabolic velocities ($v$) at two different tamoxifen concentrations. Such data are unfortunately poorly predictive of the real biotransformation in cancer or liver cells, and all discussion on tamoxifen biotransformation from such partial data may be quite unreliable.

Indeed, implication in cells of a specific P450 isozyme depends on the drug-enzyme affinity, $K_m$, and the maximal velocity of the metabolite production, $V_{max}$. From these two Michaelis-Menten constants, an intrinsic clearance can be calculated by $V_{max}/K_m$. With recombinant P450s, intrinsic clearances can be easily determined for each isozyme. Unfortunately, no $V_{max}$ and $K_m$ values are available in this article.

Moreover, it was described by Venkatarkrishnan et al. (2000) that metabolic velocities in recombinant system preparation may be strongly different from microsome preparation. Indeed, the cellular amounts of P450 coenzymes (NADPH P450 oxidoreductase and, for some isozymes, cytochrome b5) as well as lipidic membrane composition of recombinant systems (bacteria, yeast, etc.) can strongly modify P450 activity. As stated by these authors, “Use of metabolic rates ($v$) from cDNA-expressed CYPs […] may lead to incorrect prediction of the micromolar velocity […]”.

For this reason, apparent $v$ and $V_{max}$ in recombinant system preparations have to be weighted by the so-called relative activity factor (RAF; see eq. 1) before extrapolation (Venkatarkrishnan et al., 2001a,b).

When I studied the demethylation of the antidepressant, citalopram (Rochat et al., 1997), human recombinant CYP2D6 isozyme showed by far the highest production of demethyl-citalopram but had no significant contribution in vivo since poor metabolizer patients had similar pharmacokinetics to extensive ones. The high $K_m$ value of CYP2D6 partially weighted the extremely high $V_{max}$ in the determination of intrinsic clearance, but only the RAF value could have given to this isozyme its real contribution in vivo, scarce.

Therefore, identification of P450s isozymes involved in biochemical pathways can be reliable and predictive only if RAF and the intrinsic clearance are determined (see eq. 2). Today, measuring these parameters with recombinant P450s is not time consuming and gives so much more value to in vitro biotransformation data that we should not avoid their determination.

Equations 1 and 2

$$RAF = \frac{(V_{max})_{P450n \text{ in microsomes}} \times (P450n \text{ abundance})}{(V_{max})_{cDNA-expressed \text{ P450n}}}$$

$$IC = \sum \frac{(RAF \times V_{max})}{K_m} \text{ for cDNA-expressed P450n}$$

where RAF and P450n abundance are expressed as picomoles of P450n isozyme per milligram of microsomal proteins; $V_{max}$ of P450n in microsomes and $V_{max}$ of cDNA-expressed P450n are expressed as picomoles of metabolite produced per minute $\times$ picomoles of P450n isozyme. The numerator of this equation may also be expressed as picomoles of metabolite produced per minute $\times$ picomoles of microsomal proteins. $V_{max,c}$ are measured with specific probes.

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References

Response to Letter to the Editor

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We thank Dr. Rochat for his considered and thoughtful comments concerning our article (Crewe et al., 2002). However, we feel he has overestimated the scope and intended purpose of our study.

We state in the article, “The objectives of the current study were to determine which human P450 forms might participate in the extrahepatic metabolism of tamoxifen to its N-desmethyl, 4-hydroxy and 4’-hydroxy metabolites and in the isomerization of trans-4-hydroxytamoxifen.” Our objective was not to undertake a quantitative analysis of which P450s metabolize tamoxifen in vivo or to predict which forms will be more or less important. Rather we sought only to show which forms potentially may be involved, building on and extending previous in vitro studies using liver microsomes and recombinant systems (Jacquot et al., 1991; Simon et al., 1993; Williams et al., 1994; Crewe et al., 1997; Dehal and Kupfer, 1997). Indeed, given the stated aim to identify forms that might participate in extrahepatic metabolism and the paucity of information regarding the content of different P450s in extrahepatic tissues such as breast and endometrium, it is impossible at this stage to perform such a scaling procedure as suggested. This is particularly relevant when considering individual tumors, since individual P450 content is as likely to vary between different clones of cancer cells as other phenotypic characteristics. Indeed, even with liver, there is some debate as to the mean content of particular P450s. We make reference to both the importance of individual P450 expression in determining quantitative importance in vivo and the paucity of data available concerning P450 expression in many tissues. Moreover, we state, “Further studies are required . . . to determine whether and to what extent individual P450 forms may mediate tamoxifen metabolism in these tissues.”

The issue of differences between activity in recombinant systems and that in microsomes is a critical one. We allude to the fact that slight differences in environment may alter results in comparing our results regarding tamoxifen 4-hydroxylation by CYP2B6 and CYP2C19 to those of Dehal and Kupfer (1997). At present, it is not possible to quantify all the factors that may influence activity, and our own experience suggests factors such as the NADPH-cytochrome P450 reductase/P450 ratio can affect activity of the one P450 form toward alternative substrates to different extents (i.e., the RAF as defined will differ according to the substrate under consideration, since no marker substrate will fit all conditions).

Dr. Rochat also makes a valid point about the need to determine the apparent $K_m$, $V_{max}$, and intrinsic clearance for each pathway and P450 form in recombinant systems. It is only by performing a full kinetic characterization that the relative efficiencies of different forms can be compared in vitro, even before any extrapolation to the in vivo environment. Such a full characterization is clearly warranted but is not always trivial. In the case of tamoxifen, it has proven difficult to establish the necessary preliminary condition of linearity of product formation with respect to enzyme concentration at the lowest substrate concentration used, despite the absence of significant substrate depletion (Crewe et al., 1997 and unpublished data). The reasons for this are not clear and merit further investigation in light of recent studies concerning the diverse effects of protein concentration on P450 activity (Ludden et al., 1997; Carlile et al., 1999; Tang et al., 2002).

In conclusion, we reiterate that the purpose of our work was to reveal the potential for specific P450s to metabolize tamoxifen via the pathways studied and regret any unintended implication that our goal was to undertake any semi-quantitative analysis or prediction of the roles of different P450s in vivo.

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References


