Letter to the Editor

QUANTITATION OF CYP2B6, CONSTITUTIVE ANDROSTANE RECEPTOR, AND PREGNANE X RECEPTOR MRNA LEVELS

We read with great interest, an article in the January 2003 issue of Drug Metabolism and Disposition by Chang et al. (2003) that described interindividual variability and correlations between CYP2B6 mRNA expression and transcription factor mRNA expression [constitutive androstane receptor (CAR)] and pregnane X receptor (PXR) in human liver. Specifically, the paper showed that CYP2B6 mRNA and CAR mRNA from 12 livers had high interindividual variability (almost 300-fold) and correlated extremely well ($r^2 = 0.63$, $p = 0.002$). Furthermore, mRNA levels of PXR were found to correlate well with CAR and CYP2B6 mRNA levels ($r^2 = 0.86$, $p < 0.001$ and $r^2 = 0.75$, $p < 0.001$, respectively). The authors concluded that the variability in these transcription factors, especially in CAR, may contribute to the interindividual differences in CYP2B6 gene expression.

However, as an alternative explanation we propose that the observed correlations (and high variability) could equally likely have resulted from differences in mRNA quality among the 12 different human liver samples. It is well known that mRNA is very unstable and quickly degrades to different degrees, depending on collection and storage conditions. In particular, collection conditions for human tissues tend to be suboptimal. Consequently, it has become standard practice for these types of studies to normalize the data to the mRNA content of a quality control gene such as glyceraldehyde-3-phosphate dehydrogenase, hypoxanthine-guanine phosphoribosyl transferase, TATA box-binding protein, β-glucuronidase, β-actin, or 18S rRNA (Sumida et al., 1999; Rodriguez-Antona et al., 2001; Koch et al., 2002; Lin et al., 2002; Toide et al., 2002). With this approach the effect of mRNA degradation on the gene of interest should affect the normalization gene to a similar extent thereby substantially reducing associated variability. A recent article (Koch et al., 2002) suggests that 18S rRNA may be better than the other normalization genes used because there appears to be less intrinsic variability (i.e., variability resulting from true differences in gene expression) between livers.

In addition to appropriate quality controls, the use of a negative control would have helped to eliminate mRNA quality as a possible cause of the observed correlations. For example, the data could have been compared with mRNA levels for a gene that is highly unlikely to be regulated by PXR and CAR such as CYP2E1. CYP2E1 mRNA has been reported by Sumida et al. (1999) not to be correlated with CYP3A4 mRNA, a gene that like CYP2B6 is also regulated by PXR and CAR. A similar strategy was used by Toide et al. (2002), who reported that HNF-1α mRNA levels were correlated with UGT2B7 mRNA (a gene that is known to be regulated by HNF-1α), but not with UGT2B15 mRNA (a gene that is not regulated by HNF-1α).

References

1 Abbreviations used are: CAR, constitutive androstane receptor; PXR, pregnane X receptor; HNF-1α, hepatocyte nuclear factor-1α; UGT, UDP-glucuronosyltransferase.