Fractional Clearance for Verapamil N-Demethylation in the Isolated Rat Liver Preparation

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We read with great interest the manuscript by Yang et al. (Yang et al., 2015), which was published in a recent issue of the journal. The authors report the utility and advantages of an elegant PBPK model, compared with a traditional AUC approach, for the estimation of the fraction of hepatic clearance of verapamil (VER) that forms norverapamil (NOR) ($h_{mi}^{VER\rightarrow NOR}$) in an isolated perfused rat liver (IPRL) preparation. The authors graciously cite several of our prior works with verapamil in interpreting their data (Mehvar et al., 1994; Mehvar and Reynolds, 1995; Mehvar and Reynolds, 1996; Robinson and Mehvar, 1996). In particular, they cite, on multiple instances, our study on the hepatic disposition of VER and its VER-generated and preformed N-demethylated metabolite (NOR) enantiomers in an IPRL model (Mehvar et al., 1994). Unfortunately, it appears that the authors have misinterpreted and/or inaccurately cited our published data in several places in their article. In particular, they compare $h_{mi}^{VER\rightarrow NOR}$ estimated in their study with a similar parameter obtained in our report. They mistakenly state that our $h_{mi}^{VER\rightarrow NOR}$ value is 0.12, compared with their value of 0.31, and inaccurately attribute this difference to our “failure to account for sequential metabolism ($F_L^{NOR}$), the attendant nonlinearity in metabolism, and binding to red blood cells.” Additionally, they unfortunately misquote several other kinetic parameters from our study such as our free fractions of the VER enantiomers in a bovine serum albumin solution and VER hepatic intrinsic clearance, among others. These issues are clarified and addressed in more detail in the following paragraphs.

First, it is puzzling why or how the authors could have quoted a value of 0.12 for $h_{mi}^{VER\rightarrow NOR}$ from our study. In our report (Mehvar et al., 1994), we estimated the fraction of the extracted VER enantiomers converted to NOR at steady state, a parameter that should be similar to $h_{mi}^{VER\rightarrow NOR}$. Our values were clearly stated to be 0.23 and 0.19 for the S- and R-enantiomers of VER, respectively, which are in complete agreement with the value of 0.23 reported by the
authors themselves at their lowest dose for the racemic VER [Table 2 in (Yang et al., 2015)]. Additionally, our values of 0.23 and 0.19 are much closer than the misquoted value of 0.12 to their value of 0.31, which was obtained from their fitting the data to a PBPK model.

Second, the authors attribute the inaccurately quoted much lower value of $F_{mi}^{VER\rightarrow NOR}$ (0.12) to our failure to recognize sequential metabolism, nonlinearity in the metabolism of verapamil, and the binding to red blood cells, which is not an accurate interpretation of our data. In our article, we used the following equation [equation (5) in (Mehvar et al., 1994)] to estimate the fraction of infused VER enantiomer converted into its respective enantiomer of NOR during one passage through the liver at steady-state ($F_m$):

$$F_m = \frac{C_{out(NOR)}^{VER}}{C_{out(NOR)}^{NOR}} \times \frac{C_{in}^{NOR}}{C_{in}^{VER}} \quad (1)$$

where $C_{out(NOR)}^{VER}$ and $C_{out(NOR)}^{NOR}$ are the steady-state outlet concentrations of NOR after the VER and preformed NOR administrations, respectively, and $C_{in}^{VER}$ and $C_{in}^{NOR}$ are the inlet concentrations of VER and preformed NOR after their respective administrations. The estimated $F_m$ values were 0.21 and 0.18 for S- and R-VER, respectively. These values are the fractions of the administered enantiomers, and not the extracted enantiomers, which are converted to NOR in each passage through the liver. We then corrected the $F_m$ values for the extent of hepatic extraction (E) of the enantiomers to estimate fractions of the extracted enantiomers of VER converted to NOR, which were 0.23 and 0.19 for the S- and R-enantiomers, respectively. In contrast to the statement made by Yang et al. (Yang et al., 2015), the above equation does indeed consider the sequential metabolism of NOR, or what they call $F_L^{NOR}$, as demonstrated by the inclusion of the $C_{out(NOR)}^{NOR}/C_{in}^{NOR}$ ratio in the equation. Indeed, in our single-pass experiments, both VER and NOR were administered into the portal vein, which simulates oral dosing, as opposed to the administration of the bolus doses of VER and NOR into the perfusate of the
recirculating system used by Yang et al. (Yang et al., 2015), which mimics intravenous dosing, thus the additional requirement for correction by $F_L^{\text{NOR}}$ in their study. It would appear that the source of misinterpretation of our data is most likely the failure of Yang et al. (Yang et al., 2015) to recognize the differences between the addition of VER or NOR to their perfusate in their recirculating system (i.e., similar to intravenous dosing) and administration of VER or NOR into the portal inlet (i.e., similar to oral dosing) in our single-pass model. Nevertheless, these calculations assume that the metabolism of VER-generated NOR and the preformed NOR, administered as such, are similar. Additionally, these calculations assume linear pharmacokinetics. Our inlet concentrations of VER enantiomers were ~1.2 µM, which were within two-fold of the lowest racemic concentration of 1 µM used by Yang et al. (Yang et al., 2015) and far from their high saturating concentrations of 50 and 100 µM. Nevertheless, we clearly acknowledged these limitations by stating in our manuscript that “because of assumptions inherent to this type of calculation (20), the $F_m$ values obtained here should be considered only as estimates of the actual values,” with reference (20) in our original article referring to the work of the corresponding author in the manuscript by Yang et al. (Pang and Kwan, 1983).

As for the “failure to account for...binding to red blood cells,” the following equation, which is a modified version of eq. (1) used in our manuscript, accounts for the red blood cell binding of VER and NOR by inclusion of blood: plasma ratios of VER [(B:P)$_{\text{VER}}$] and NOR [(B:P)$_{\text{NOR}}$]:

$$F_m = \frac{C_{\text{VER}}^{\text{NOR}} \times (B:P)_{\text{NOR}}}{C_{\text{out}(\text{NOR})}^{\text{NOR}} \times (B:P)_{\text{NOR}}} \times \frac{C_{\text{NOR}}^{\text{VER}} \times (B:P)_{\text{VER}}}{C_{\text{in}}^{\text{VER}} \times (B:P)_{\text{VER}}}$$  \hspace{1cm} (2)

The above equation is simplified to the following equation:

$$F_m = \frac{C_{\text{VER}}^{\text{NOR}}}{C_{\text{out}(\text{NOR})}^{\text{NOR}}} \times \frac{C_{\text{NOR}}^{\text{VER}}}{C_{\text{in}}^{\text{VER}}} \times (B:P)_{\text{VER}}$$  \hspace{1cm} (3)
which suggests that our reported values based on equation (1) should have been corrected for the 
\((B:P)_{\text{NOR}}/(B:P)_{\text{VER}}\) ratio. However, we used only a 10% red blood cell in our study (Mehvar et al., 1994), and the 
\((B:P)_{\text{NOR}}\) and \((B:P)_{\text{VER}}\) values were very similar to each other for both 
enantiomers as demonstrated by us in another IPRL study that used 20% RBC (Mehvar and Reynolds, 1996). Therefore, in contrast to the suggestion by Yang et al. (Yang et al., 2015), a 
lack of consideration of the red blood cell uptake in our studies is unlikely to have affected the 
differences, if any, between our \(F_m\) values and their \(h^{\text{VER} \rightarrow \text{NOR}}\) values.

Lastly, the authors inaccurately cite a number of parameters from our study. For example, 
they state that our unbound fractions of R-VER and S-VER in a 2% bovine serum albumin were 
\(~0.65\) and \(~0.55\), respectively. However, as our data (Fig. 3 in (Mehvar et al., 1994)] clearly 
show, we reported free fractions of 0.23 and 0.12, respectively. Furthermore, they cite a value of 
50-130 ml/min from our study for total hepatic intrinsic clearance of VER \(\left(CL_{\text{int,tot}}^{\text{VER}}\right)\), whereas in 
reality our values were 260 and 430 ml/min (Table I, in (Mehvar et al., 1994)].

In conclusion, the fractions of VER converted to its metabolite NOR in our single-pass 
IPRL (0.23 and 0.19 for the S- and R-enantiomers, respectively) are in complete agreement with 
the value reported by Yang et al. when a similar method, based on the AUCs of VER and 
generated and preformed NOR, was used to estimate this parameter in a recirculating IPRL after 
a relatively low dose of VER. However, when the authors used the maximum velocity and 
Michaelis-Menten parameters of VER and NOR metabolism in a PBPK model to estimate this 
parameter, a higher value (0.31) was obtained (Yang et al., 2015). Considering the significant 
variability (CVs of 60-80%) in the estimates of \(h^{\text{VER} \rightarrow \text{NOR}}_{\text{mi}}\) reported by Yang et al. [Tables 2 and 
5 in (Yang et al., 2015)], it is hard to argue for a marked difference between the traditional AUC 
method at a low VER dose and the PBPK model for the estimation of this parameter.
References


