Letter to the Editor

Probenecid-Associated Alterations in Valproic Acid Pharmacokinetics in Rats: Can in Vivo Disposition of Valproate Glucuronide Be Predicted from in Vitro Formation Data?

I read with interest the recent article by Ward et al. (2000) on the interactions between probenecid and valproic acid in rats. The authors attempted to predict the in vivo disposition of valproate glucuronide in the presence of probenecid using in vitro metabolism experiments and concluded that the in vitro prediction approach was not successful in this case. Although the authors provided two hypotheses as possible explanations (effects of probenecid on translocation of valproate glucuronide from the hepatocyte into blood and on the oxidative metabolism of valproate), it may be important to consider a few other explanations.

In the in vivo infusion studies, probenecid coadministration caused a significant decrease in the systemic clearance of valproate and this was attributed to competitive inhibition of valproate glucuronide formation by probenecid, based solely on an in vitro study in rat liver S9. There are two caveats in drawing this inference. First, data from these two studies by themselves do not necessarily establish a cause-effect relationship between valproate glucuronidation rate, in vitro, and the systemic clearance of valproic and/or its glucuronide metabolite, in vivo, because probenecid cannot only affect glucuronidation but also oxidative metabolism and is known to be a substrate for many transporters, as the authors have acknowledged. Second, although the Kᵢ for the inhibition of valproate glucuronide formation, in vitro, by probenecid in this study was 876 μg/ml, the target total plasma concentration of probenecid in the in vivo study was only 50 to 75 μg/ml (plasma probenecid concentrations were not determined in this study), i.e., less than one-tenth the Kᵢ value. The unbound probenecid concentrations in plasma and presumably in the cytosol were probably less than 1% of the in vitro Kᵢ, because probenecid is highly bound to serum proteins (Vree et al., 1993). Inhibition of valproate glucuronide formation in vivo under those conditions would therefore be unlikely, i.e., lack of any change in the valproate glucuronide biliary excretion by probenecid in this study is only to be expected. Furthermore, the calculated valproate glucuronide biliary excretion rate in the absence of probenecid treatment does not show clear concentration dependence (see Fig. 4 in Ward et al., 2000), which may suggest that the processes involved in the hepatobiliary disposition of this substrate are nearly saturated at the concentrations of valproate glucuronide employed in this study and in turn would preclude the possibility of observing a discernible effect of any inhibitor of those processes.

This study serves to underline the difficulties involved in studying the in vitro-in vivo correlation for drug interactions when the substrate or the inhibitor modulate multiple ADME (absorption, distribution, metabolism, and excretion) processes and the need to design such studies with agents that exhibit a high degree of selectivity to the pathways in question.

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References

Response to Letter to the Editor

We would like to thank Dr. Yeleswaram for his review of, and comment on, our recent publication (Ward et al., 2000). In particular, we appreciate the fact that his letter reinforces the primary theme of this study, i.e., predictions of in vivo drug interactions based on in vitro metabolic data require a comprehensive understanding of all pharmacokinetic processes that dictate the disposition of the agent of interest. While we agree with Dr. Yeleswaram’s conclusion, we feel that it is necessary to clarify some points of potential confusion in his commentary.

In contrast to Dr. Yeleswaram’s contention, we drew no “cause-effect” conclusions by comparing the in vivo (decreased intrinsic clearance of valproate in the presence of probenecid) and in vitro (increased Kᵢ for valproate glucuronidation in the presence of probenecid) data. In fact, we simply stated that the results of the two experiments were “consistent”, which indeed they are. We were careful to state that probenecid “competitively inhibits the elimination” of valproate precisely because we recognized that the two agents may interact at multiple sites or via multiple processes (only one of which, glucuronidation, was examined in vivo). This is an especially important point, as it is the propensity for multiple interactions between two agents (valproate and probenecid in this case) that obscures in vitro/in vivo relationships.

Although it is true that the target probenecid concentrations in this experiment (50–75 μg/ml) were well below the in vitro Kᵢ for inhibition of valproate glucuronide formation (876 μg/ml), one must always consider the uncertainty inherent in model-generated kinetic parameters. The coefficient of variation associated with determination of the in vitro Kᵢ for inhibition of valproate glucuronidation was 63%, suggesting that the actual Kᵢ in a given rat liver may have been substantially lower than this model-derived estimate. Moreover, careful consideration of the in vitro metabolism data provided in Table 3 reveals that the Kᵢ for valproate glucuronidation was increased approximately 2-fold even at low probenecid concentrations (14.3 μg/ml). Although Dr. Yeleswaram states correctly that probenecid is highly bound to proteins in human serum (Vree et al., 1993), protein binding in rat serum is more modest (Emanuelsson and Paulzow, 1989). In addition, it is important to remember that probenecid uptake into the hepatocyte is mediated by a saturable, inhibitable process, a phenomenon that has been recognized for over three decades (Gigon and Guarino, 1970). In fact, despite the moderate binding of probenecid in rat serum, the liver-to-serum (total) probenecid concentration ratio is approximately 2 at 60 min after a 50-mg/kg dose of probenecid (Guarino and Schanker, 1968). Thus, we might reasonably expect hepatic probenecid concentrations exceeding 100 μg/ml, which should decrease the formation clearance of valproate glucuronide >2-fold (Ward et al., 2000; Table 3). If inhibition of valproate glucuronide formation was the only mechanism by which valproate and probenecid interacted, then we should have observed a decrease
in the fraction of the valproate dose excreted in bile as the glucuronide conjugate. The mass balance of valproate glucuronide was unaffected by probenecid, despite the fact that probenecid 1) inhibits glucuronide formation in vitro, 2) decreases the systemic clearance of valproate in vivo, and 3) should have been present in vivo at sufficient hepatic concentrations to decrease glucuronide formation. This observation is a clear indication that the interaction involved processes in addition to formation of valproate glucuronide.

Finally, Dr. Yeleswaram observed a lack of concentration dependence in the relationship between biliary valproate glucuronide excretion and concentrations of the parent drug in hepatic cytosol (Fig. 4), and suggested that the apparent absence of concentration dependence may indicate that the processes governing hepatobiliary disposition of the conjugate were nearly saturated (presumably even at the lowest valproate concentration). This suggestion is inconsistent with available data concerning the disposition of valproate and its glucuronide conjugate in rats. For example, as initial valproate concentrations increased from approximately 60 to 1200 μg/ml, the net contribution of biliary excretion of the glucuronide conjugate to overall valproate clearance (reflecting the contributions of both glucuronide formation and biliary excretion of the conjugate) decreased from 40% to <20% (Liu et al., 1993). The majority of the saturability in valproate/valproate glucuronide disposition was encountered at serum valproate concentrations exceeding 250 μg/ml. In Ward et al. (2000), unbound cytosolic valproate concentrations did not exceed the in vitro $K_m$ for glucuronidation. Although saturation of valproate glucuronide transport is conceivable, no measurable valproate glucuronide was detected in hepatic cytosol, even at the highest valproate infusion rate. Because systemic concentrations of valproate glucuronide did not increase disproportionately with valproate infusion rate, and because no valproate glucuronide was detected in hepatic cytosol, saturation of valproate glucuronide transport cannot explain the mass balance results obtained in this study. In interpreting data such as those presented in Fig. 4, it is important to realize that the excretion of a metabolite is plotted relative to the concentration of the parent compound. Several intervening steps (formation of the metabolite, intracellular binding, intracellular translocation to the excretory site, and excretory transport of the metabolite) must occur for the metabolite to actually appear in bile. Given the relatively small number of animals examined in this experiment, the absence of a clear concentration relationship in this complicated system is neither surprising nor problematic.

Agents that inhibit specific metabolic or transport pathways are useful in probing mechanisms of drug interactions, as Dr. Yeleswaram suggests. However, a more practical issue is the ability of in vitro systems to accurately predict potential in vivo drug interactions. This latter topic has received considerable attention over the past several years, particularly in the context of new drug development. The probenecid/valproate interaction serves to underscore the point that accurate predictions of in vivo interactions from in vitro data are possible only when the complete spectrum of processes governing the disposition of a compound is understood.

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References


