What is the value of studying mycophenolic acid (MPA) glucuronidation in the esophagus? Whereas the authors would consider the esophagus not worthy of study viz. MPA bioavailability, our stated aim was to carry out in vitro characterization of the primary metabolizers of mycophenolic acid among human recombinant UDP-gluco- nosyltransferases (UGTs), especially those distributed in gastrointestinal tissues. It is noteworthy that a small amount of an unusually high activity enzyme could have an impact in any tissue, and there are potentially second-pass pharmacokinetic effects. These factors could apply to UGT1A7, which is highly concentrated in the esophagus with less in the remaining gastrointestinal tissues (Basu et al., 2004a).

Thin-layer chromatography (TLC) is a poor analytical tool? Although high-pressure liquid chromatography is preferred for analysis of products present in trace or low amounts, radioactive MPA-glucuronide formation with [14C]UDP-glucuronic acid (0.012 μCi/nmol) by enzymes that reach saturation kinetics between 1.6 and 2.4 mM MPA followed by TLC resolution easily overcomes this objection. We describe base hydrolysis to remove acylglucuronide according to Shipkova et al. (with MPA-7-O-glucuronide analyzed by HPLC) compared with our [14C]MPA-7-O-glucuronide resolved by TLC with quantitation of radioactivity. Moreover, β-glucuronidase hydrolysis confirms that the products are glucuronides (Fig. 3B). We also cite studies by other investigators who analyzed radiolabeled glucuronides following similar TLC resolution (Mackenzie, 2000; Martineau et al., 2004).

Why carry out differential pH measurements on UGT isozymes? Our aim was to conduct a biochemical study of the individual isozymes using optimal in vitro conditions. The overlapping curves allow the reader to draw his/her own conclusions over a pH range. Although some investigators prefer the “physiological pH condition” for in vitro studies, the common practice is the determination of optimal pH condition(s) for in vitro studies.

What is the meaning of atypical kinetics? We consider it appropriate and reasonable to use “atypical metabolism” and “atypical increases in activity with increasing concentrations” to describe the results in this study. We have not used “atypical kinetics” in this report. We believe that the high rate of MPA glucuronide formation that increases with MPA concentrations up to millimolar levels described in this study likely contributes to the high dosage requirement. This phenomenon was not known before this report. One can argue, therefore, that the obvious is not necessarily true: high in vivo MPA-glucuronide concentrations cause high dosage requirement. Furthermore, it is not understood why MPA-glucuronides accumulate to such high levels in the blood, adversely affecting free MPA blood levels? What about transporters. . . etc. etc.? Although the pharmacokinetics of MPA-glucuronide clearance from the blood has likely been addressed, it has not been easy to locate that aspect of the literature? What is the significance of a number of in vivo related points with respect to our results? It is not clear that the authors are justified in attempting to pose a number of in vivo related questions that we have not posed or addressed in such an in vitro study. It is not certain that the authors’ extrapolations to the existing literature can answer all their questions. Most likely, specific experiments are required. It is noted that inhibition by curcumin (Fig. 4B, Basu et al., 2004b) represents mouse in vivo data which warrant further investigation.

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References

ABBREVIATIONS: MPA, mycophenolic acid; UGT, UDP-glucuronosyltransferase; TLC, thin-layer chromatography.

Received September 15, 2004; accepted September 16, 2004
Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.
doi:10.1124/dmd.104.002345.