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COMMENTARY

Does CYP2E1 play a major role in the aggravation of isoniazid toxicity by rifampicin in human hepatocytes?

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Isoniazid and rifampicin are first-line anti-tubercular drugs. In a recent paper, Shen *et al.* provided interesting findings that rifampicin exacerbated isoniazid toxicity in human hepatocytes but not in rat hepatocytes. The main conclusion was that the difference in cytochrome P450 2E1 (CYP2E1) induction by rifampicin between rat and human hepatocytes accounted for the difference in exacerbation of isoniazid hepatotoxicity by rifampicin. 4-Nitrophenol hydroxylase (4-NP) activity was the only probe of CYP2E1 activity used in the paper. The authors presented data showing that rifampicin enhanced 4-NP activity and CYP2E1 mRNA expression in human hepatocytes, but not in rat hepatocytes. However, CYP3A also makes a significant contribution to 4-NP activity in humans and rats, which has been confirmed by both CYP3A-specific inducer and inhibitors. Rifampicin is a strong inducer of human CYP3A; thus, the increase in 4-NP activity in human hepatocytes could be due to the induction of CYP3A. Rifampicin did not increase 4-NP activities in rat hepatocytes, which could reflect a lack of the induction of rat CYP3A by rifampicin. Additionally, more experiments are needed to support the conclusion that rifampicin increased CYP2E1 mRNA expression in human hepatocytes because of the small sample size and the limitations of semi-quantitative RT-PCR. The study by Shen *et al.* suggests that another drug-metabolizing enzyme rather than CYP2E1 could be involved in the aggravation of isoniazid toxicity by rifampicin in human hepatocytes.
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The 2008 paper by Shen *et al.* is available from http://www3.interscience.wiley.com/cgi-bin/fulltext/121667602/PDFSTART

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Isoniazid and rifampicin are the first-line drugs for the treatment of tuberculosis. As isoniazid and rifampicin are frequently given together to patients, hepatotoxicity is the major concern during the treatment with these drugs. Cytochrome P450s (CYPs) are responsible for the biotransformation of xenobiotics and endogenous compounds. The regulation of CYPs is the most common mechanism that can lead to drug–drug interactions (Kalgutkar *et al.*, 2007). Cytochrome P450 2E1 (CYP2E1) produces free radicals independent of a ligand, which can cause cell damage from lipid peroxidation and DNA strand breaks (Caro and Cederbaum, 2004). Previous studies suggest that hepatic CYP2E1 plays an essential role in isoniazid-induced hepatotoxicity through generation of free radicals (Huang *et al.*, 2003; Yue *et al.*, 2004; Shen *et al.*, 2006).

In a recent paper, Shen *et al.* (2008) provided interesting findings that rifampicin exacerbated isoniazid toxicity in human hepatocytes but not in rat hepatocytes. The main conclusion was that a difference in CYP2E1 induction by rifampicin between rat and human hepatocytes accounted for the difference in exacerbation of isoniazid hepatotoxicity by rifampicin. This was derived from the observation that rifampicin enhanced 4-nitrophenol hydroxylase (4-NP) activity and CYP2E1 mRNA expression in human hepatocytes, but not in rat hepatocytes. 4-NP was the only probe of CYP2E1 activity used in the paper; however, the specificity of 4-NP is questionable. Earlier work has revealed that another CYP, CYP3A, also makes a significant contribution to 4-NP activity in humans and rats (Zerilli *et al.*, 1997; Zerilli *et al.*, 1998; DiPetrillo *et al.*, 2002; Kobayashi *et al.*, 2002). The role of CYP3A in 4-NP activity was confirmed by CYP3A-specific inducers and inhibitors in intact rats and rat hepatocytes. Dexamethasone is a potent inducer of CYP3A, but not CYP2E1, which can significantly increase hepatic CYP3A protein and CYP3A1 mRNA in rats. The activity of 4-NP was markedly increased in dexamethasone-treated rats, which can be inhibited up to 50% by the specific CYP3A inhibitors, ketoconazole and troleandomycin. Rifampicin is a strong inducer of human CYP3A, which was mentioned in the discussion by Shen *et al.* (2008). Thus, the induction of CYP3A by rifampicin could be responsible for the increase in 4-NP activity in human hepatocytes. Previous studies have reported

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that there are species differences in the effects of rifampicin on hepatic CYP3A. In humans and dogs, rifampicin is a potent inducer of CYP3A, but not in rat and mouse, whereas dexamethasone can strongly induce CYP3A in both human and rat (Graham *et al*., 2002; Martignoni *et al*., 2004; Vignati *et al*., 2004; Nishimura *et al*., 2007). Rifampicin did not increase 4-NP activities in rat hepatocytes, which could be due to the lack of induction of rat CYP3A by rifampicin. This further supports the hypothesis that the induction of human CYP3A by rifampicin would contribute to the increase in 4-NP activity in human hepatocytes.

Another finding by Shen *et al*. (2008) was that rifampicin increased CYP2E1 mRNA expression in human hepatocytes. In the paper, the hepatocytes for mRNA expression experiment were derived from one donor. Semi-quantitative conventional RT-PCR instead of real-time RT-PCR was used for the measurement, and the times for the repeating experiments have not been mentioned. There are some inconsistent reports that the levels of CYP2E1 mRNA and protein were not increased by rifampicin in human hepatocytes (Mattes and Li, 1997; Rae *et al*., 2001; Raucy *et al*., 2004). Because of the small sample size and the limitations of the technical approach, we consider that no firm conclusion can be drawn from this finding.

Does CYP2E1 play a major role in the aggravation of isoniazid toxicity by rifampicin in human hepatocytes? CYP2E1 shows a strong conservation among species (Martignoni *et al*., 2006). Rat is thought to be a good experimental model of CYP2E1-mediated metabolism, despite some discrepancies (rat CYP2E1 shares 80% identity to human CYP2E1) (Lieber, 1999; Morel *et al*., 1999; Zuber *et al*., 2002; Martignoni *et al*., 2006). The extrapolation for CYP2E1 between species appears to be fairly good. In contrast, the rat is not a good model of CYP3A4-dependent metabolism (Zuber *et al*., 2002; Martignoni *et al*., 2006). Shen *et al*. (2008) found that rifampicin did not induce 4-NP activities in rat hepatocytes and that there was no exacerbation of isoniazid toxicity by the addition of rifampicin. This is consistent with the *in vivo* results that rifampicin co-administration failed to aggravate isoniazid hepatotoxicity after 21 day treatment by the attenuation of isoniazid-induced CYP2E1 activities (Yue *et al*., 2004). The study by Shen *et al*. (2008) suggests that another drug-metabolizing enzyme rather than CYP2E1 could be involved in the aggravation of isoniazid toxicity by rifampicin in human hepatocytes.

In summary, although the finding for the differential effect of rifampicin on isoniazid toxicity in human and rat hepatocytes is certainly welcome, the experiments do not support a clear conclusion that the discrepancy results from a simple difference in CYP2E1 regulation by rifampicin. This paper highlights the difficulties inherent in the choice of both the enzyme probes for catalytic activity studies and the most relevant animal species to humans. Particular care is needed when comparing data on drug metabolism between species.

References

Caro AA, Cederbaum AI (2004). Oxidative stress, toxicology, and pharmacology of CYP2E1. *Annu Rev Pharmacol Toxicol* **44**: 27–42.

DiPetrillo K, Wood S, Kostrubsky V, Chatfield K, Bement J, Wrighton S *et al*. (2002). Effect of caffeine on acetaminophen hepatotoxicity in cultured hepatocytes treated with ethanol and isopentanol. *Toxicol Appl Pharmacol* **185**: 91–97.

Graham RA, Downey A, Mudra D, Krueger L, Carroll K, Chengelis C *et al*. (2002). *In vivo* and *in vitro* induction of cytochrome P450 enzymes in beagle dogs. *Drug Metab Dispos* **30**: 1206–1213.

Huang YS, Chern HD, Su WJ, Wu JC, Chang SC, Chiang CH *et al*. (2003). Cytochrome P450 2E1 genotype and the susceptibility to antituberculosis drug-induced hepatitis. *Hepatology* **37**: 924–930.

Kalgutkar AS, Obach RS, Maurer TS (2007). Mechanism-based inactivation of cytochrome P450 enzymes: chemical mechanisms, structure-activity relationships and relationship to clinical drug-drug interactions and idiosyncratic adverse drug reactions. *Curr Drug Metab* **8**: 407–447.

Kobayashi K, Urashima K, Shimada N, Chiba K (2002). Substrate specificity for rat cytochrome P450 (CYP) isoforms: screening with cDNA-expressed systems of the rat. *Biochem Pharmacol* **63**: 889–896.

Lieber CS (1999). Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998) – a review. *Alcohol Clin Exp Res* **23**: 991–1007.

Martignoni M, de Kanter R, Grossi P, Mahnke A, Saturno G, Monshouwer M (2004). An *in vivo* and *in vitro* comparison of CYP induction in rat liver and intestine using slices and quantitative RT-PCR. *Chem Biol Interact* **151**: 1–11.

Martignoni M, Groothuis GM, de Kanter R (2006). Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin Drug Metab Toxicol* **2**: 875–894.

Mattes WB, Li AP (1997). Quantitative reverse transcriptase/PCR assay for the measurement of induction in cultured hepatocytes. *Chem Biol Interact* **107**: 47–61.

Morel G, Cossec B, Lambert AM, Binet S (1999). Evaluation of rat hepatic 2E1 activity in function of age, sex and inducers: choice of an experimental model capable of testing the hepatotoxicity of low molecular weight compounds. *Toxicol Lett* **106**: 171–180.

Nishimura M, Koeda A, Suganuma Y, Suzuki E, Shimizu T, Nakayama M *et al*. (2007). Comparison of inducibility of CYP1A and CYP3A mRNAs by prototypical inducers in primary cultures of human, cynomolgus monkey, and rat hepatocytes. *Drug Metab Pharmacokinet* **22**: 178–186.

Rae JM, Johnson MD, Lippman ME, Flockhart DA (2001). Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. *J Pharmacol Exp Ther* **299**: 849–857.

Raucy JL, Lasker J, Ozaki K, Zoleta V (2004). Regulation of CYP2E1 by ethanol and palmitic acid and CYP4A11 by clofibrate in primary cultures of human hepatocytes. *Toxicol Sci* **79**: 233–241.

Shen C, Zhang H, Zhang G, Meng Q (2006). Isoniazid-induced hepatotoxicity in rat hepatocytes of gel entrapment culture. *Toxicol Lett* **167**: 66–74.

Shen C, Meng Q, Zhang G, Hu W (2008). Rifampicin exacerbates isoniazid-induced toxicity in human but not in rat hepatocytes in tissue-like cultures. *Br J Pharmacol* **153**: 784–791.

Vignati LA, Bogni A, Grossi P, Monshouwer M (2004). A human and mouse pregnane X receptor reporter gene assay in combination with cytotoxicity measurements as a tool to evaluate species-specific CYP3A induction. *Toxicology* **199**: 23–33.

Yue J, Peng RX, Yang J, Kong R, Liu J (2004). CYP2E1 mediated isoniazid-induced hepatotoxicity in rats. *Acta Pharmacol Sin* **25**: 699–704.

Zerilli A, Ratanasavanh D, Lucas D, Goasduff T, Dreano Y, Menard C *et al*. (1997). Both cytochromes P450 2E1 and 3A are involved in the O-hydroxylation of p-nitrophenol, a catalytic activity known to be specific for P450 2E1. *Chem Res Toxicol* **10**: 1205–1212.

Zerilli A, Lucas D, Dreano Y, Picart D, Berthou F (1998). Effect of pyrazole and dexamethasone administration on cytochrome P450 2E1 and 3A isoforms in rat liver and kidney: lack of specificity of p-nitrophenol as a substrate of P450 2E1. *Alcohol Clin Exp Res* **22**: 652–657.

Zuber R, Anzenbacherova E, Anzenbacher P (2002). Cytochromes P450 and experimental models of drug metabolism. *J Cell Mol Med* **6**: 189–198.