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COMMENTARY

Alcohol-Mediated Increases in Acetaminophen Hepatotoxicity

ROLE OF CYP2E AND CYP3A

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ABSTRACT. This commentary focuses on the roles of CYP3A and CYP2E in alcohol-mediated increases in acetaminophen hepatotoxicity. CYP2E has been considered to be the main form of P450 responsible for such toxicity in animals and humans. However, CYP3A, which is also induced by alcohol, has been shown to have a greater affinity for acetaminophen than CYP2E. Previous experiments implicating CYP2E in alcohol-mediated increases in acetaminophen hepatotoxicity have used inhibitors of this form of P450 that are now proving to be non-specific. Triacetyloleandomycin (TAO) is a potent inhibitor of CYP3A that maintains specificity *in vitro* over a large concentration range. In rats treated with ethanol or the combination of ethanol and isopentanol, the major higher chain alcohol in alcoholic beverages, TAO protects animals from increases in acetaminophen hepatotoxicity, suggesting a major role of CYP3A. CYP2E may not have a major role due to the rapid loss of induced levels in the absence of continued exposure to ethanol. Knockout mice, which are being used to define the role of particular proteins in biological responses, have been developed for CYP2E1 and CYP1A2 but not CYP3A. *Cyp2e1(−/−)* and *Cyp1a2(−/−)* mice are more resistant to acetaminophen hepatotoxicity than wild-type strains, even though the amounts of the other forms of P450s are unaltered in the liver. These findings suggest that the relative amounts of P450s and not just kinetic characteristics determine their role in acetaminophen hepatotoxicity. The clinical implications of the findings that CYP3A can have a major role in acetaminophen-mediated hepatotoxicity are discussed. BIOCHEM PHARMACOL **55**;10:1557–1565, 1998. © 1998 Elsevier Science Inc.

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The observation that alcoholics are susceptible to developing liver damage from therapeutic or otherwise nontoxic doses of acetaminophen was first reported in South Africa in 1977 [1]. Several other case reports were published during the late 1970s and early 1980s (for review, see [2]), including reports of alcoholics who experienced hepatotoxicity or even death from liver failure after reportedly receiving therapeutic doses of acetaminophen [3, 4]. In rodents, pretreatment with ethanol, the major alcohol in alcoholic beverages [5, 6], was first reported to increase acetaminophen hepatotoxicity in 1980 [7]. This finding has been confirmed in several experimental systems (for review, see [8]). However, until very recently, concern over potential interactions between alcohol and acetaminophen resulting in hepatotoxicity has been limited to the alcoholic patient.

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INVOLVEMENT OF CYTOCHROME P450 IN THE FORMATION OF THE REACTIVE METABOLITE OF ACETAMINOPHEN: ROLE OF CYP2E

The pioneering work of Mitchell and coworkers [9] showed that P450-mediated metabolism of acetaminophen is responsible for the hepatotoxicity of this drug. *N*-Acetylbenzimidoquinone was subsequently identified to be a major toxic metabolite of acetaminophen, formed by cytochrome P450 [10] and also by peroxidases [10, 11] and prostaglandin synthase [12]. At least three separate forms of cytochrome P450 are capable of converting acetaminophen to *N*-acetylbenzimidoquinone, namely CYP2E, CYP3A, and CYP1A2 [13–17]. All of these forms have been detected in varying amounts in human liver [17–19]. Hepatic CYP2E can be induced up to eight-fold by ethanol in rodents [20] and is elevated in association with consumption of alco-

holic beverages in humans [21, 22]. Thus, CYP2E has been considered the main form of cytochrome P450 responsible for alcohol-mediated increases in acetaminophen hepatotoxicity in animals and humans [2, 8, 23, 24]. A major role of CYP2E is supported by the findings that several inhibitors of CYP2E protect against acetaminophen hepatotoxicity [25–27] and that inducers of CYP2E other than ethanol, such as isoniazid, also increase acetaminophen hepatotoxicity [27, 28]. However, as discussed in more detail below, several inhibitors of CYP2E are proving to be nonspecific, and that inducers, once considered specific for CYP2E, are now also found to induce CYP3A.

INDUCTION OF CYP3A BY THE PREDOMINANT ALCOHOLS IN ALCOHOLIC BEVERAGES

Ethanol is considered to be a classic inducer of CYP2E (for review, see Ref. 29). However, ethanol has also been shown to induce CYP3A in several experimental systems, including primary cultures of rat hepatocytes [30], intact rats [31, 32], and a rat hepatoma cell line [33]. Although most investigations into the effect of consumption of alcoholic beverages on drug metabolism and acetaminophen hepatotoxicity have focused on ethanol [2, 8, 29], other alcohols can also be present in these beverages, with isopentanol constituting the major higher chain alcohol in most alcoholic beverages [5, 6]. The amount of isopentanol can be as high as 0.23 to 0.5%, depending on the type of beverage as well as the preparation [5, 6]. In rodents and avians, ethanol and isopentanol have been shown to increase barbiturate-inducible forms of cytochrome P450, in addition to inducing CYP2E [30–37]. In rats, although isopentanol alone is a poor inducer of hepatic CYP3A and CYP2E, combined treatment with ethanol and isopentanol can result in synergistic increases in CYP3A [31]. In rats, at least five forms of CYP3A have been identified thus far [38]. The antibody used in our studies recognizes many forms of CYP3A. Using specific oligonucleotide probes that distinguish CYP3A2 from CYP3A1 mRNA, the alcohols increased CYP3A2 mRNA and not CYP3A1 mRNA [31]. The oligonucleotide probe used to measure CYP3A1 [31] also recognizes CYP3A23, a form of CYP3A now identified to be the major form of CYP3A induced in rats by barbiturates [39]. Therefore, the alcohols do not induce CYP3A23. Because, in rats, a number of chemicals are reported to induce different forms of CYP3A [39–42], it may be important to compare the relative abilities of these different forms to activate acetaminophen in order to assess their role in acetaminophen hepatotoxicity.

The isopentanol content of alcoholic beverages may have a major role in induction of CYP3A and aggravation of acetaminophen hepatotozicity associated with consumption of alcoholic beverages in humans. In primary cultures of human hepatocytes, ethanol and isopentanol each induce immunoreactive CYP2E and CYP3A, the increase in CYP3A being associated with an increase in CYP3A4 mRNA [43]. In these cultures, isopentanol is a more potent inducer than ethanol, not only for CYP2E but also for CYP3A [43]. Hoshino and Kawasaki [44] reported that consumption of alcoholic beverages elevates CYP3A in humans. The noninvasive method used by these authors to measure CYP3A involves determining the urinary ratio of 6β-hydroxycortisol to 17-hydroxycortisol, rather than to total cortisol in a 24-hr urinary sample. They found that the urinary levels of 17-hydroxycortisol show less day-to-day variation than cortisol, and are not altered by alcoholic liver disease. In 27 alcoholic patients admitted to the hospital for detoxification, the ratio of 6β-hydroxycortisol to 17-hydroxycortisol in urine taken over the first 24 hr after admission was twice the ratio observed in normal volunteers. The alcoholic patients showed a 50% decrease in these values after 2 weeks of detoxification, suggesting that CYP3A had been increased in these patients by the consumption of alcoholic beverages and had then declined during the detoxification period. Recent findings on the metabolism of fentanyl provide indirect support for the ability of alcoholic beverages to induce CYP3A in humans. In alcoholic patients, the dose of fentanyl required to achieve analgesia is higher than in non-alcoholic patients [45]. Two recent studies have found that CYP3A is the major form of cytochrome P450 responsible for fentanyl metabolism by human liver microsomes [46, 47]. If fentanyl resistance in alcoholic patients is found to result from increased metabolism, this will support the possibility that CYP3A is induced by alcohol consumption in these patients. In conclusion, because the results for rodents, human hepatocytes, and humans indicate that alcohols induce CYP3A, it is important to determine whether CYP3A has a role in alcohol-mediated increases in acetaminophen hepatotoxicity.

COMPARISON OF CYP3A AND CYP2E IN THE ACTIVATION OF ACETAMINOPHEN

Kinetic analysis reveals that the affinity of CYP3A for acetaminophen is considerably greater than that of CYP2E [16, 17]. This suggests that, CYP3A is more likely to be responsible for most acetaminophen bioactivation at the low tissue levels of acetaminophen associated with therapeutic doses of this drug, except when the CYP2E content is far greater than that of CYP3A. The finding that CYP3A has a lower K_m than CYP2E for conversion of acetaminophen to *N*-acetylbenzimidoquinone has been reported by two independent laboratories [16, 17]. The actual K_m values varied, however, depending on the system of investigation. Using purified, reconstituted human CYP3A, the K_m for acetaminophen was found to be 0.14 mM [17], a value close to plasma levels of patients taking acetaminophen therapeutically [48]. Unfortunately, this study did not include an analysis of the kinetics for acetaminophen activation by reconstituted, purified human CYP2E. However, another study did evaluate purified, reconstituted human CYP2E, and found a K_m for acetaminophen of 4.2

mM [16], a value similar to the K_m of 5.9 mM reported for rabbit CYP2E [13]. Interestingly, the affinity of the human CYP2E for acetaminophen increased in the presence of cytochrome b_5 (K_m of 0.61 mM), as did the activity. With purified rat CYP2E [49] and expressed human CYP3A4 [16], inclusion of cytochrome b_5 resulted in increased activity. Thus, the activity of both CYP2E and CYP3A may be enhanced in the intact liver by cytochrome b_5.

A study investigating the metabolism of acetaminophen in intact lymphoblastoma cells expressing CYP2E1 [50] obtained a K_m for acetaminophen of 0.18 mM, which is as low as values reported for CYP3A, but far lower than values reported for CYP2E (see above). It is unfortunate that these authors did not perform similar studies using lymphoblastoma cells expressing CYP3A for comparative purposes. Some caution is needed in interpreting kinetic values in experiments using intact cells. The V_{max} value for acetaminophen activation was considerably lower in the lymphoblastoma cells expressing CYP2E compared with values obtained using either reconstituted CYP2E or microsomes from HepG2 cells transfected with human CYP2E [16]. In measuring the metabolism of a substrate by intact cells, the rate of metabolism may be either limited or enhanced by other factors in these cells, which cannot be easily controlled or identified.

With hepatic microsomes isolated from rat [16] and human [17] liver, acetaminophen activation exhibits biphasic kinetics. In hepatic microsomes from rats treated with dexamethasone, however, only the low K_m (0.056 mM) activity was evident [16]. Because dexamethasone induces CYP3A in rodents [40, 41] with no concomitant increase in CYP2E [30], the low K_m probably represents CYP3A. Studies with microsomes from rats treated with ethanol yielded two K_m values for acetaminophen, one of 0.037 mM and another of 0.915 mM. Both the low K_m and the high K_m activities were induced by ethanol [16] and may represent rat CYP3A and CYP2E, respectively.

One means of assessing overall activity is to compare the ratio of V_{max} to K_m. Assuming, in hepatic microsomes from ethanol-treated rats, that the low K_m activity and its associated V_{max} for acetaminophen activation represent the kinetics for CYP3A, while the high K_m activity and its associated V_{max} represent CYP2E [16], the ratios of V_{max}/K_m are similar for CYP3A and CYP2E. The ratios of V_{max}/K_m are also similar for human forms of CYP3A and CYP2E expressed in HepG2 cells [16]. These data indicate that both CYP3A and CYP2E can contribute to acetaminophen activation and hepatotoxicity, the exact contribution depending on the relative amounts of these P450s in the liver.

Large variations in the amounts of CYP3A and CYP2E have been found in human livers [17–19]. In microsomal samples from four different human livers, the contribution of CYP3A to acetaminophen metabolism, as determined by the extent of inhibition obtained with antibodies to CYP3A, varied from 6 to 76% and correlated with the amount of CYP3A [16]. In another study, where the contribution of CYP3A was evaluated in four liver samples using TAO*, a specific inhibitor of CYP3A, [51, 52], the extent of inhibition varied from 1 to 20% [17]. In other studies, the contribution of CYP3A was found to be less when TAO-mediated inhibition of activity was used as a measure of CYP3A compared with antibody-mediated inhibition of activity [16, 53]. Inhibition of CYP3A by TAO may be less complete than antibody inhibition and, therefore, may underestimate the actual contribution of CYP3A. However, a large number of human liver samples must be analyzed for the relative contribution of CYP2E and CYP3A to acetaminophen activation before the overall contribution of CYP3A can be assessed.

USE OF INHIBITORS *IN VIVO* TO DEFINE THE ROLE OF CYP3A AND CYP2E IN ALCOHOL-MEDIATED INCREASES IN ACETAMINOPHEN HEPATOTOXICITY

One approach to determine the role of a particular form of P450 in drug toxicity involving bioactivation is to investigate whether treatment with a specific inhibitor of that form protects animals from the toxicity. A major problem with this approach is that inhibitors administered *in vivo* may prove less specific than when used *in vitro*, due to the very high concentrations often achieved in the liver of the intact animal. In addition, some inhibitors are dissolved in solvents, such as DMSO and polyethylene glycol, which themselves inhibit certain forms of P450 [54–56].

TAO, however, is a potent inhibitor of CYP3A [51, 52] that maintains specificity *in vitro* over a large concentration range [52]. TAO protects rats from increases in acetaminophen hepatotoxicity, associated with pretreatment with either ethanol alone [57, 58] or with the combination of ethanol plus isopentanol (Sinclair J, Szakacs J, Jeffery E, Kostrubsky V, Wood S, Wright D, Wrighton S and Sinclair P, unpublished results). These findings suggest that CYP3A plays a major role in alcohol-mediated increases in acetaminophen hepatotoxicity. In these experiments, we administered TAO in saline instead of DMSO, because DMSO itself may prevent acetaminophen hepatotoxicity by inhibiting CYP2E [54, 55] or by decreasing oxidative damage [59].

The protection afforded by TAO varied with the kind of alcohol treatment and the dose of acetaminophen. In rats pretreated with the combination of ethanol and isopentanol, a massive increase in acetaminophen hepatotoxicity was observed as early as 7 hr after administration of doses of acetaminophen as low as 0.5 g/kg [60] or even 0.25 g/kg (Sinclair J, Szakacs J, Jeffery E, Kostrubsky V, Wood S, Wright D, Wrighton S and Sinclair P, unpublished results). At the lower acetaminophen dose, there was almost complete protection by TAO, as indicated by a decrease in serum levels of AST and ALT, as well as prevention of

* *Abbreviations*: ALT, alanine aminotransferase; AST, aspartate aminotransferase; 4MP, 4-methylpyrazole; and TAO, triacetyloleandomycin.

histological damage. In contrast, at the higher dose of acetaminophen, there was only partial protection afforded by TAO (Sinclair J, Szakacs J, Jeffery E, Kostrubsky V, Wood S, Wright D, Wrighton S and Sinclair P, unpublished results). The results suggest that either the inhibition of CYP3A by TAO was incomplete at the higher acetaminophen dose or that CYP3A is not solely responsible for acetaminophen bioactivation at this dose and CYP2E may have a role.

In rats pretreated with ethanol alone, TAO completely protects animals from histologically-observed liver damage associated with administration of high doses of acetaminophen (1 g/kg), but does not prevent the elevation in serum levels of AST [57]. The sustained elevation in serum levels of AST may represent (1) lack of complete inhibition of CYP3A by TAO, (2) the contribution of CYP2E, or (3) some effect of the alcohols other than induction of P450 that contributes to the liver damage. Alternatively, AST may not be liver-specific, and elevations in this enzyme may reflect damage to another organ.

In many studies, the accepted major role of CYP2E in acetaminophen hepatotoxicity has been determined using inhibitors that were considered to be specific for CYP2E. However, many of these inhibitors have now been shown to be less specific than previously thought. For example, diethyldithiocarbamate, previously regarded as a specific inhibitor of CYP2E, is an even better inhibitor of CYP3A [51]. 4-MP, an inhibitor of CYP2E, also inhibits other forms of cytochrome P450, including CYP3A [52]. 4-MP protects phenobarbital-pretreated rats from acetaminophen hepatotoxicity [27], a pretreatment that increases CYP3A and CYP2B [29], but decreases CYP2E [34]. Although DMSO inhibits CYP2E [54, 55] and protects rodents from acetaminophen hepatotoxicity [26], DMSO also quenches hydroxyl radicals [61], which may contribute to the oxidative damage associated with acetaminophen hepatotoxicity [8]. Park *et al.* [59] found that DMSO decreases the hepatotoxicity of acetaminophen in mice, without decreasing the covalent binding *in vivo* of acetaminophen to protein, and suggested that the protection occurs via the scavenging of free radicals. Diallyl sulfide has been reported to specifically inhibit CYP2E *in vitro* [62] and to protect against acetaminophen hepatotoxicity *in vivo* in otherwise untreated rodents [25, 63]. These results suggest a major role for CYP2E in acetaminophen hepatotoxicity in the uninduced rat. However, diallyl sulfide also inhibits CYP3A and CYP1A *in vitro*, although not as potently as CYP2E [63]. In addition, no investigations have been made, as yet, into the effect of diallyl sulfide on alcohol-mediated increases in acetaminophen hepatotoxicity.

As is well known, ethanol itself inhibits CYP2E [64]. When administered simultaneously with acetaminophen, ethanol protects rodents against acetaminophen hepatotoxicity [65–68], findings regarded as evidence for the involvement of CYP2E. However, consumption of alcoholic beverages simultaneously with drug administration results in decreased metabolism and clearance of a wide variety of drugs, many of which are known to be metabolized by pathways not involving CYP2E [69]. In rats, the protection afforded by ethanol against acetaminophen-mediated liver damage occurs at a plasma ethanol concentration of only 5 mM [68]. A concentration of 5 mM of ethanol has no effect on acetaminophen activation by rat or human microsomes [64, 68]. Therefore, this concentration of ethanol does not act to competitively inhibit P450. *In vivo*, ethanol may inhibit acetaminophen activation by an indirect mechanism such as causing transient decreases in hepatic levels of NADPH [68, 70, 71]. Alternatively, hepatic concentrations of ethanol at the site of P450 may be far higher than in the plasma.

Regardless of the specificity of inhibitors demonstrated *in vitro*, in all of the studies, including our own, it must eventually be demonstrated, using substrate-specific metabolism *in vivo*, that the dose of an inhibitor used in intact animals does not alter its specificity. Using this approach requires identification of a substrate that is specific *in vivo* for the form of P450 being analyzed. The N-demethylation of erythromycin [72] and the 1-hydroxylation of midazolam [73] have been used as biomarkers for CYP3A in humans, while the 6-hydroxylation of chlorzoxazone has been used as a biomarker for CYP2E [74]. However, chlorzoxazone is also metabolized by CYP3A [75] and CYP1A2 [76]. Although *p*-nitrophenol has been used as a specific substrate of CYP2E [77], we find that its hydroxylation is increased in microsomes from dexamethasone-treated rats, and this activity is inhibited by TAO, indicating an involvement of CYP3A (Chatfield K, Sinclair J, Wood S, Sinclair P, Jeffery E and Wrighton S, unpublished results).

EFFECT OF WITHDRAWAL FROM ALCOHOL ON HEPATIC LEVELS OF CYP3A AND CYP2E

A major complication in experimental studies investigating the effect of ethanol pretreatment on acetaminophen hepatotoxicity is the effect of withdrawal from ethanol on the hepatic levels of CYP2E and CYP3A before the administration of acetaminophen. This withdrawal is used in order to eliminate ethanol from the blood, since, as mentioned above, plasma ethanol concentrations of only 5 mM are sufficient to protect animals from acetaminophen hepatotoxicity [68]. In most studies investigating alcohol-mediated increases in acetaminophen hepatotoxicity, animals are removed from the alcohol-containing diet 16–24 hr before administration of acetaminophen [7, 65, 66, 78]. After this withdrawal period, the alcohol-induced CYP2E declines to levels observed in the untreated animal [32, 79]. In contrast, alcohol-mediated increases in CYP3A are sustained during this withdrawal period [32]. Thus, in many experimental protocols used to investigate ethanol-mediated increases in acetaminophen hepatotoxicity, CYP3A may assume a relatively greater role than CYP2E due to the loss of induced CYP2E in the absence of continued exposure to ethanol. In our rat experiments, although there was some loss of CYP2E following ethanol removal 11 hr prior

to acetaminophen administration, CYP2E was still elevated compared with that from rats not treated with ethanol [57]. The protection afforded by TAO in these animals suggests that, even in the presence of elevated levels of CYP2E, CYP3A is responsible for most of the alcohol-mediated increases in acetaminophen hepatotoxicity.

ACETAMINOPHEN HEPATOTOXICITY IN MICE GENETICALLY ENGINEERED TO BE DEFICIENT IN CYP2E1 OR CYP1A2

Another approach to identify the role of a specific form of cytochrome P450 in the metabolism of drugs and the activation of carcinogens and hepatotoxins involves knocking out the gene for that specific form of P450, and then investigating the effect on the metabolism, carcinogenicity, or hepatotoxicity of the chemical in question. Of the three forms of P450 most active in converting acetaminophen to the hepatotoxic metabolite, "knockout" mice have been developed for two of these forms, CYP2E1 [80] and CYP1A2 [81, 82]. The CYP2E1 knockout mouse [*Cyp2e1(−/−)*] is found to be more resistant to acetaminophen hepatotoxicity than the wild-type mouse, indicating that CYP2E1 has an important role in acetaminophen hepatotoxicity [80]. However, *Cyp1a2(−/−)* mice are also resistant to acetaminophen hepatotoxicity [83], even though hepatic levels of CYP2E1 and CYP3A are not affected by the *Cyp1a2* deletion [84]. These latter findings suggest that, despite its high K_m and low V_{max} for acetaminophen [16], CYP1A2 may have an essential role in acetaminophen hepatotoxicity even when CYP2E and CYP3A are present in the liver. In a recent study comparing *Cyp1a2(−/−)* and *(+/+)* mice, two parameters attributed to acetaminophen bioactivation and toxicity, namely hepatic thiol depletion and covalent binding of acetaminophen to proteins, were similar after a 2-hr exposure to acetaminophen [85]. These results suggest that CYP1A2 does not have a major role in acetaminophen bioactivation, consistent with the kinetic analyses of human and rat P450s [15, 16]. However, the results are inconsistent with the findings that the *Cyp1a2(−/−)* mice are more resistant than the wild-type mice, to acetaminophen hepatotoxicity [83], even though CYP2E and CYP3A are not affected by the deletion [84]. Although thiol depletion and covalent binding of acetaminophen to protein in the liver are used as indicators of acetaminophen bioactivation, they do not always correlate with the extent of liver damage [8, 60].

One possible explanation for the difference between the findings in the hepatotoxicity study [83] and the findings in the study measuring the binding of acetaminophen to protein [85] is the length of time that the animals were exposed to acetaminophen. In the study comparing acetaminophen hepatotoxicity in the *Cyp1a2(−/−)* and *(+/+)* mice, the animals were exposed to acetaminophen for 24 hr [83], while in the study analyzing the binding of acetaminophen to protein, the time of exposure was only 2 hr. Over 2 hr, a fractional increase in acetaminophen bioactivation in the *Cyp1a2(+/+)* mouse compared with the *Cyp1a2(−/−)* mouse may prove inconsequential. However, over a 24-hr exposure, this difference may be sufficient to account for the greater hepatotoxicity observed in the *Cyp1a2(+/+)* mouse [83]. A second possibility for the different responses observed in the two studies may be a function of the dose–response. Dose–response studies with acetaminophen in mice show a threshold dose at which toxicity occurs [86]. This threshold dose varies not only between mouse strains, but also within same inbred strain, including C57BL/6 [87–89], which is part of the genetic background of the *Cyp1a2(−/−)* mouse [82]. To assess the relative roles of CYP1A2, CYP2E1, and CYP3A in acetaminophen hepatotoxicity using knockout mice, the sensitivity to acetaminophen must not only be compared in the same genetic background [90], but a dose–response study may also be needed with each batch of mice to compare hepatotoxicity with other parameters of acetaminophen bioactivation. In addition, in the studies in which both the *Cyp2e1(−/−)* [80] and *Cyp1a2(−/−)* [83] mice were found to be resistant to acetaminophen hepatotoxicity, there may be an additional alteration in these knockout mice that makes them more resistant to acetaminophen-mediated hepatotoxicity. Each of these possibilities suggests that caution should be taken in interpreting results with knockout mice as unequivocal evidence for the involvement of a particular form of P450. The relative amounts of CYP1A2, CYP3A, and CYP2E in the liver may prove important, in addition to their kinetic parameters, when assessing the contribution of these forms of CYP450 to acetaminophen hepatotoxicity.

CONCLUSIONS

In rats, two experimental findings suggest that CYP3A plays a significant role in alcohol-mediated increases in acetaminophen hepatotoxicity: 1) TAO, a specific inhibitor of CYP3A, protects animals from alcohol-mediated increases in acetaminophen hepatotoxicity [57, 58]; and 2) in most published studies investigating the effect of alcohol pretreatment on acetaminophen hepatotoxicity, CYP2E has decreased to control levels by the time of administration of acetaminophen, while the alcohol-mediated increases in CYP3A are sustained [57]. The kinetic parameters of CYP3A, CYP2E, and CYP1A2 for acetaminophen activation *in vitro* [16] suggest that, at equimolar amounts, CYP3A will have greater activity than CYP2E, and both forms will be more active than CYP1A2. However, the resistance of the *Cyp1a2(−/−)* mouse to acetaminophen [83], despite wild-type levels of CYP2E and CYP3A [84], indicates that CYP1A2 can also play a significant role in acetaminophen hepatotoxicity. The relative roles of CYP2E, CYP3A, and CYP1A2 in alcohol-mediated increases in acetaminophen hepatotoxicity may be clarified by investigating whether alcohol induces CYP3A and increases acetaminophen hepatotoxicity in *Cyp2e1(−/−)* and *Cyp1a2(−/−)* mice. Further research is necessary to

determine whether the alcohols have other actions in the liver, independent of P450, that contribute to the increased acetaminophen hepatotoxicity, as suggested in studies with mice [66], or whether increases in CYP2E and CYP3A are the sole causes for this increased toxicity. There is need to clarify the relative role of CYP1A2 since, in humans, cigarette smoking [18] and consumption of barbecued meat [91] can result in elevated levels of CYP1A2 in the liver and may contribute to increased acetaminophen hepatotoxicity in combination with consumption of alcoholic beverages.

A role for CYP3A in acetaminophen hepatotoxicity in humans would have important clinical implications. A number of drugs increase CYP3A levels [29] and treatment with these drugs may increase acetaminophen hepatotoxicity. Some medications contain caffeine as well as acetaminophen. Caffeine and plant flavonoids activate CYP3A and increase acetaminophen hepatotoxicity in certain experimental systems [92, 93], and possibly in humans. In experimental animals, fasting increases both CYP3A [94, 95] and CYP2E [34], decreases hepatic glutathione levels and increases acetaminophen hepatotoxicity [96]. Therefore, loss of weight, due to dieting or in diseases such as cancer, may increase the risk of developing acetaminophen hepatotoxicity in humans. It is interesting to note that weight loss in normal individuals [97] and patients with AIDS [98] has been identified recently as a possible risk for acetaminophen hepatotoxicity.

Another concern is that CYP3A may be destroyed during acetaminophen bioactivation [57]. Because CYP3A is responsible for the metabolism of many drugs, the clinical implications for acetaminophen-induced loss of CYP3A are significant, as has already been noted by others [17]. Drugs normally cleared by CYP3A metabolism might, therefore, accumulate following administration of acetaminophen.

In summary, there is now substantial evidence that alcohols induce not only CYP2E but also CYP3A. Furthermore, CYP3A appears to be involved in alcohol-mediated increases in acetaminophen hepatotoxicity in rats. At the time that CYP2E was first identified as having a major role in ethanol-mediated increases in acetaminophen hepatotoxicity, the kinetics for activation of acetaminophen by CYP3A had not yet been characterized. In addition, the possibility exists that other forms of cytochrome P450 or other enzymes, such as cyclooxygenase, will be found to contribute to ethanol enhancement of acetaminophen toxicity. A thorough investigation of the role of CYP3A will be necessary to determine if CYP3A lies at the root of acetaminophen hepatotoxicity in the context of consumption of alcoholic beverages.

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