Contribution of CYP2E1 and CYP3A to acetaminophen reactive metabolite formation

Background: CYP2E1, 1A2, and 3A4 have all been implicated in the formation of *N*-acetyl-*p*-benzoquinone imine (NAPQI), the reactive intermediate of acetaminophen (INN, paracetamol), in studies in human liver microsomes and complementary deoxyribonucleic acid–expressed enzymes. However, recent pharmacokinetic evidence in humans has shown that the involvement of CYP1A2 is negligible in vivo. The purpose of this study was to evaluate the respective roles of CYP2E1 and 3A4 in vivo.

Methods: The involvement of CYP2E1 was assessed through pretreatment of adult human volunteers with disulfiram to inhibit the enzyme and the role of CYP3A4 through its induction in a second cohort of adults with rifampin (INN, rifampicin). Each of the respective studies was an open-label, balanced-randomized crossover design. Blood samples were obtained serially for 12 hours and urine was collected for 24 hours after acetaminophen administration. Acetaminophen was assayed in plasma, and acetaminophen and metabolites were assayed in urine.

Results: The recovery of the thiol metabolites formed by conjugation of NAPQI with glutathione was decreased by 69%, and the formation clearance of NAPQI was decreased by 74% (both $P < .01$) by pretreatment with disulfiram. Rifampin pretreatment had no effect on the formation of NAPQI or the recovery of thiol metabolites formed by conjugation of NAPQI with glutathione.

Conclusions: CYP2E1 accounts for the formation of NAPQI in intact humans; the contribution of other isozymes of cytochrome P450 appears to be negligible. Under some conditions, disulfiram may be useful in diminishing the formation of NAPQI after acetaminophen overdose. (Clin Pharmacol Ther 2000;67:275-82.)

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Acetaminophen (INN, paracetamol), a widely used over-the-counter mild analgesic and antipyretic drug, causes liver necrosis when large doses are ingested, putatively through the formation of *N*-acetyl-*p*-benzoquinone imine (NAPQI).[1] The predominant pathways of acetaminophen biotransformation are direct formation of glucuronide and sulfate conjugates. There are two microsomal cytochrome P450–catalyzed oxidative pathways of acetaminophen metabolism, one forming NAPQI and the other a catechol (3-hydroxy) metabolite. Each of these products of oxidation is eliminated in the urine as several daughter metabolites. All NAPQI products eliminated in the urine are formed through conjugation of NAPQI with glutathione and are referred to as thiol metabolites.[2]

Several members of the cytochrome P450 superfamily of oxidases have been shown to form NAPQI. The first to be identified was CYP2E1; this enzyme is responsible for the generation of electrophilic species from several small molecular weight molecules. CYP1A2 and CYP3A4 have also been shown, based on studies in human liver microsomes or complementary deoxyribonucleic acid (cDNA)–expressed enzymes, to produce NAPQI from acetaminophen.[3-5] The quantitative contribution of these various isozymes of cytochrome P450 toward NAPQI formation in vivo has not been determined. In addition, there is evidence to

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suggest that contributions of cytochrome P450 enzymes determined in microsomes may not reflect the in vivo situation. For example, recent pharmacokinetic data in healthy volunteers pretreated with omeprazole for 7 days showed that induction of CYP1A2 did not enhance NAPQI formation after acetaminophen administration to CYP2C19-deficient subjects.[6] Induction of CYP1A2 in this study was confirmed by a 75% increase in caffeine clearance.

It has been previously inferred that CYP2E1 is important in the formation of NAPQI in humans in vivo as a result of drug interaction studies with isoniazid and ethanol.[7-9] Both of these agents are established inducers of CYP2E1.[10,11] In addition, isoniazid first inhibits and then enhances NAPQI formation, which is consistent with the mechanism of induction by ligand stabilization.[9] This cytochrome P450 induction mechanism is apparently specific (from lack of evidence for any other isoform) to CYP2E1.

Use of a potent and highly selective mechanism-based inhibitor provides direct proof of the participation of an enzyme in the formation of a specific metabolite in the native uninduced state and is an indication of its quantitative importance.[12] Single-dose disulfiram, through the reduced disulfiram metabolite diethyldithiocarbamate, has been identified as a selective mechanism-based inhibitor of rat and human liver microsomal CYP2E1, both in vitro and in vivo.[13] Recent studies have shown that single-dose disulfiram administration to humans inhibits CYP2E1 activity by approximately 95% but does not affect the activity of CYPs 2A6, 2C9, 2C19 2D6, or 3A4.[14,15] Single-dose disulfiram is therefore an appropriate tool to assess the contribution of CYP2E1 to the formation of NAPQI in humans.

In vitro kinetic and inhibition studies in human liver microsomes by Patten et al[4] with anti-CYP3A4 antibodies and troleandomycin, a specific CYP3A4 inhibitor, have suggested that CYP3A4 (Michaelis-Menten constant [K_m] = 300 μmol/L) could contribute appreciably (44% to 49%) to NAPQI formation at therapeutically relevant acetaminophen concentrations (100 μmol/L) in vivo.[16] Conversely, troleandomycin caused a 10% inhibition of NAPQI formation by human liver microsomes at 100 μmol/L acetaminophen in studies conducted in our laboratory.[5] At concentrations associated with acetaminophen toxicity, 2 to 10 mmol/L (corresponding to a 20-g dose), troleandomycin inhibited NAPQI formation by 4% to 5%. Our laboratory results therefore suggest that CYP3A4 is a minor contributor to NAPQI formation in vivo (15% at most). A direct assessment of the contribution of CYP3A4 to NAPQI formation in humans in vivo has not been reported.

The antibiotic rifampin (INN, rifampicin), widely used for the treatment and prophylaxis of tuberculosis (often in combination with isoniazid), is perhaps the most potent inducer of CYP3A4 in clinical use.[17] However, rifampin is not a specific CYP3A4 inducer. Other P450s induced by rifampin include CYP2C9, CYP2C19, and CYP1A2, none of which appears to contribute significantly to NAPQI formation.[18] Because rifampin is taken for up to 6 months at a time and acetaminophen is used ubiquitously, it is very likely that the two drugs may be used together. Indeed, because isoniazid and rifampin are commonly combined, it is possible that rifampin contributes to the enhanced toxicity of acetaminophen observed in patients receiving antituberculotic drug treatment.[19] We therefore chose to use rifampin induction to determine whether CYP3A4 contributes appreciably to NAPQI formation in vivo and to assess its possible role in potentiating acetaminophen toxicity.

The purpose of this investigation was to establish the contribution of uninduced human CYP2E1 to the formation of NAPQI and to determine whether disulfiram might be a useful inhibitor of NAPQI formation in cases of acetaminophen overdose. In addition, we examined the effects of rifampin on the formation of NAPQI in humans to assess the potential risk that induction of CYP3A4 presents for acetaminophen-induced hepatic necrosis. We regard induction of CYP3A4 as a more powerful paradigm than inhibition to assess a contribution to NAPQI formation because CYP3A4 is thought to be a minor contributor in the uninduced state.

MATERIAL AND METHODS

Chemicals. Analytical grade acetaminophen and β-glucuronidase type H-2 were purchased from Sigma Chemical Co (St Louis, Mo). All other chemicals and solvents were of reagent grade or better.

Human subjects. Sixteen healthy volunteers participated in each of two (disulfiram and rifampin, respectively) randomized and open-label crossover studies. The studies were approved by the University of Washington Human Subjects Review Committee, and all subjects provided written informed consent. Subjects were in good health (assessed by physical examination and liver function tests), had no history of liver or kidney disease, and were not taking medication during the course of the investigation. The study was limited to subjects within 15% of ideal body weight who were nonsmokers and who drank alcohol infrequently or not at all. Female subjects were not pregnant. The study population comprised students (age range, 23 to 35

years) from the University of Washington campus, with equal numbers of men and women. They were instructed to refrain from drinking alcohol or ingesting any medication for 1 week before, during, and for another week after administration of disulfiram. Subjects abstained from caffeine and grapefruit juice the day before each study day and throughout the period of blood and urine collection.

Study design. Subjects were separately enrolled into disulfiram or rifampin studies. Each study was an open-label crossover design, with subjects randomly assigned to the control or treatment (disulfiram or rifampin) phase. The crossover phase was initiated 2 to 3 weeks after the first administration of acetaminophen. Subjects fasted overnight and emptied their bladders just before taking acetaminophen at approximately 8:30 AM. No food was ingested for another 2 hours. Thereafter, subjects were allowed free access to food and water. For the control phases of each study, subjects received 500 mg acetaminophen (McNeil Consumer Products) orally with water at 8:30 AM. In the disulfiram phase, each subject received 500 mg disulfiram (Wyeth-Ayerst) orally at bedtime, approximately 10 hours before acetaminophen (500 mg) administration. In the rifampin phase, each subject received 600 mg rifampin (Marion Merrell Dow) orally at bedtime for a week, with the last dose given approximately 10 hours before acetaminophen (500 mg) administration. Blood was collected through a 20-gauge indwelling catheter inserted into an arm or hand vein of each subject. Ten milliliters of baseline blood was collected just before acetaminophen administration. Blood samples were collected over sodium ethylenediaminetetraacetic acid (EDTA) at 0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after acetaminophen administration. Plasma was separated by centrifugation and stored at –20°C for later analysis. Total urine output for the first 24 hours after acetaminophen administration was collected over 3 g ascorbic acid. Urine was maintained at 4°C throughout the collection period. The total urine produced was recorded, and a 15-mL aliquot was stored at –20°C until analysis.

Analytical methods. Acetaminophen and metabolites in plasma and urine were assayed by a modification of the reversed-phase HPLC method as described previously.[20,21] For measurement of acetaminophen in plasma, 200 μL of the sample was transferred to a clean polypropylene tube and an equal volume of 1.0 mol/L perchloric acid was added to precipitate protein. The mixture was vortexed briefly and centrifuged at 13,000*g* for 5 minutes. Two hundred microliters of the supernatant was transferred to a second clean polypropylene tube and neutralized with an equal volume of 0.7 mol/L dibasic potassium phosphate, pH 11 (adjusted to pH 11 with 10 mol/L potassium hydroxide). This mixture was cooled in an ice bath for 30 minutes, and then centrifuged for 5 minutes. Fifty microliters of the supernatant was injected onto a 10-cm reversed-phase C_{18} column (3 μm; Microsorb-MV, Rainin, Woburn, Mass) that was maintained at room temperature. Acetaminophen eluted with a retention time of 6.7 minutes under a tertiary gradient mobile phase that consisted of acetonitrile, methanol, and buffer (20 mmol/L monobasic ammonium phosphate/acetate, 0.1%, vol/vol, pH 3.1). Ultraviolet absorbance was monitored at 254 nm. The initial mobile phase was 5% methanol/buffer. Methanol was increased linearly from 5% to 8% over 10 minutes and then to 9% over a minute. While the methanol concentration remained at 9%, the acetonitrile concentration was increased from 0% to 5% over a minute and then increased to 30% over the next minute, maintained for another minute, decreased to 5% (together with methanol) over another minute, and finally decreased to 0% over another minute. The column was re-equilibrated for 2 minutes with the initial mobile phase conditions.

Acetaminophen concentration in plasma was quantitated in duplicate from a standard curve that was linear ($r^2 = 0.995$) over the range of 0.125 to 10 μg/mL, with an interday coefficient of variation of <10% at 0.125 μg/mL. Acetaminophen in urine was also assayed by the method described above. Acetaminophen, acetaminophen sulfate, and acetaminophen glucuronide were quantitated from unhydrolyzed urine. Two hundred microliters of urine was added to 600 μL of 0.2 mol/L sodium acetate/acetic acid buffer (pH 5.0) and filtered through a 10 kDa molecular weight cutoff membrane (Alltech, Deerfield, Ill) by centrifugation at 13,000*g* for 5 minutes. Five microliters of the supernatant was injected onto the HPLC column, and the glucuronide, sulfate, and acetaminophen were eluted with retention times of 4.5, 6.3, and 8.4 minutes, respectively. Total urine recovery of acetaminophen and quantitation of the fraction of the dose recovered as the 3-hydroxyacetaminophen, 3-methoxyacetaminophen, and the cysteinyl-, mercapto-, and methylthio-conjugates of acetaminophen were determined. Five hundred microliters of the urine sample was added to the same volume of 0.125 mol/L sodium acetate buffer (pH 5.0) that contained 40 μL of β-glucuronidase/sulfatase and incubated overnight at 37°C. Four hundred microliters of the hydrolyzed urine was filtered as described above. Ten microliters of the supernatant was injected onto the HPLC column. The hydroxy-, cysteinyl-, methoxy-, mercapto-, and methylthio-conjugates were eluted with retention times of 3.7, 5.4, 12.6, 15.2, and 20.3 minutes, respectively.

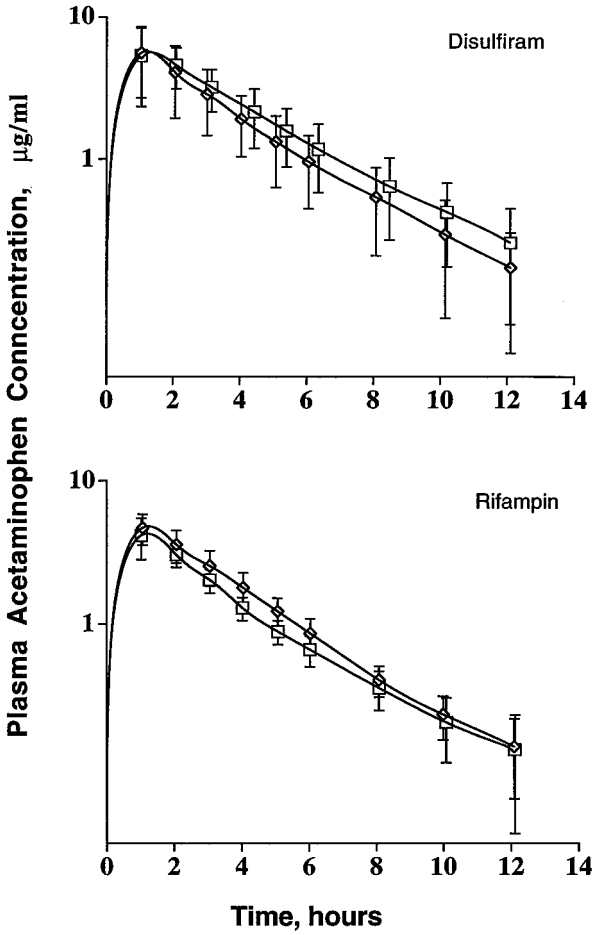

Fig 1. Plasma concentration–time profile of acetaminophen for all subjects. **Top panel,** Disulfiram study. **Bottom panel,** Rifampin study. Each *point* represents the mean value of eight subjects. *Diamonds* and *squares* are control and treatment phases, respectively. *Bars* represent SD.

Because several acetaminophen metabolites are chemically unstable during long-term storage and are hygroscopic, quantitation was achieved by determination of an ultraviolet extinction coefficient for each metabolite of interest. ^{14}C-Acetaminophen of known specific activity was administered to a mouse, and 24-hour urine was collected. Combined ultraviolet and radioactivity detection of acetaminophen metabolites eluting from the HPLC column generated a relative response factor for each metabolite, recorded as disintegrations per minute and hence, molar amount per ultraviolet peak area unit. Response was linear, with interday coefficients of variation for the urine analysis less than 5%.

Pharmacokinetic analysis. Plasma clearance of acetaminophen was calculated as the ratio of dose/AUC, in which AUC is the total area under the plasma concentration–time curve. Half-life ($t_{½}$) and AUC were calculated by WinNonlin (Pharsight, Palo Alto, Calif). Maximum acetaminophen concentration in plasma and the time of its occurrence were determined by direct inspection of the data (not from model-based interpolations). The fraction of acetaminophen dose recovered as each respective metabolite in 24-hour urine was calculated as the product of metabolite concentration in urine and the 24-hour urine volume divided by the 500-mg dose ingested. The formation clearance of each metabolite was calculated as the product of plasma clearance and the fraction of acetaminophen dose recovered in urine as that metabolite.

Statistics. Results are expressed as mean values ± SD. The Student paired *t* test was used to assess significant differences between control and treatment groups, with $\alpha = .05$.

RESULTS

Fig 1 represents the mean acetaminophen plasma concentration–time profile for the eight subjects in each respective study. The effects of disulfiram and rifampin treatments on peak plasma acetaminophen concentration, clearance, and elimination $t_{½}$ for individual subjects are given in Table I. Disulfiram pretreatment decreased plasma acetaminophen clearance by 18% ($P < .05$). This was accompanied by a 10% prolongation of the elimination $t_{½}$ ($P < .05$). There was no change in peak plasma concentration or time to peak concentration caused by disulfiram. Rifampin pretreatment significantly increased plasma acetaminophen clearance by 21% ($P < .05$) but had no effect on peak plasma concentration, time to peak concentration, or elimination $t_{½}$.

Urine recoveries of acetaminophen and its metabolites and the formation clearance of the respective metabolites are shown in Table II. Mean recovery of the acetaminophen dose was complete and unaffected by disulfiram or rifampin treatment. Glucuronidation and sulfation were the major routes of acetaminophen elimination; the thioether metabolites (those formed through the initial conjugation of NAPQI with glutathione) accounted for less than 10% of the dose. Disulfiram increased the urinary recovery of unchanged acetaminophen by approximately 25% and that of its glucuronide conjugate by 11% ($P < .05$). The recovery of the sulfate conjugate of acetaminophen was not changed by pretreatment with disulfiram. In contrast, the recovery of all oxidative metabolites was decreased by disulfiram pretreatment. Recovery of the acetaminophen catechol metabolite was decreased by approximately 29% ($P = .01$). Recovery of the thioether daughters of

Table I. Effect of treatment with disulfiram or rifampin on acetaminophen pharmacokinetic parameters

Subject No.	*Weight (kg)*	*Sex*	*CL (mL/kg/h)*		*Elimination $t_{½}$ (h)*		*C_{max} (μg/mL)*		*t_{max} (h)*	
			Control	*Treatment*	*Control*	*Treatment*	*Control*	*Treatment*	*Control*	*Treatment*
Disulfiram										
1	87	Male	528	385	2.23	2.40	3.38	4.07	1.12	1.00
2	55	Female	297	215	2.52	2.86	8.21	9.30	1.00	1.22
3	47	Female	285	355	2.26	2.31	11.4	8.94	1.10	1.07
4	47	Female	523	403	2.03	2.44	6.07	6.43	1.00	1.07
5	53	Female	424	440	1.93	2.19	5.66	4.73	1.97	2.23
6	69	Male	431	351	2.57	2.48	3.69	5.09	1.00	1.03
7	100	Male	579	365	1.61	1.63	3.24	4.68	1.08	2.15
8	95	Male	440	345	1.91	2.42	3.59	4.16	1.03	1.05
Mean±SD	69.1±22.0		438±106	358±65.7	2.13±0.32	2.34±0.35	5.65±2.90	5.93±2.10	1.16±0.33	1.35±0.52
P Value				.04		.03		.58		.2
Rifampin										
1	58	Female	435	472	1.78	2.17	6.33	5.46	1.17	0.98
2	84	Male	357	385	2.41	2.67	4.53	4.18	1.08	1.02
3	58	Female	362	588	2.04	1.63	6.54	5.47	1.08	1.05
4	76	Female	412	424	1.92	1.99	4.46	4.56	1.15	1.00
5	86	Male	389	576	2.18	2.29	4.46	2.80	1.00	2.07
6	69	Female	378	445	2.09	1.80	4.20	4.98	1.00	1.00
7	95	Male	427	528	1.82	2.03	3.39	3.13	1.07	1.07
8	70	Male	425	490	2.79	2.74	3.71	3.95	1.00	1.13
Mean±SD	74.5±13.3		404±39.6	489±71.7	2.13±0.34	2.17±0.39	4.70±1.14	4.32±1.00	1.07±0.07	1.17±0.37
P Value				.02		.73		.21		.52

Statistical comparisons were made with the Student paired *t* test.
CL, Clearance; $t_{½}$, half-life; C_{max}, peak plasma concentration; t_{max}, time to reach C_{max}.

Table II. Effect of disulfiram or rifampin on the recovery of acetaminophen and its metabolites in urine

Product	*Urinary recovery as a percentage of dose*		
	Control	*Treatment*	*P Value*
Disulfiram			
Acetaminophen	2.20 ± 1.24	2.75 ± 1.31	.04
Acetaminophen glucuronide	54.2 ± 7.93	60.2 ± 10.3	.03
Acetaminophen sulfate	28.0 ± 8.81	30.5 ± 11.4	.27
Catechol	5.96 ± 1.24	4.23 ± 1.31	.01
Thiols	9.11 ± 5.03	2.80 ± 0.75	.01
TOTAL	99.5 ± 8.53	100 ± 7.36	.82
Rifampin			
Acetaminophen	3.30 ± 0.79	2.77 ± 0.63	.01
Acetaminophen glucuronide	48.8 ± 9.71	47.3 ± 7.47	.55
Acetaminophen sulfate	32.9 ± 6.69	31.0 ± 6.58	.13
Catechol	6.47 ± 1.76	6.83 ± 2.01	.66
Thiols	8.81 ± 2.32	8.11 ± 2.09	.39
TOTAL	100 ± 10.9	96.0 ± 4.28	.26

Data reported are mean values ± SD. Statistical comparisons were made with the Student paired *t* test.

NAPQI was diminished by 69% (P = .01). Rifampin decreased the urinary recovery of unchanged acetaminophen (from 3.30% ± 0.79% to 2.77% ± 0.63%; P = .01). With the exception of the catechol metabolites, urinary metabolite recovery tended to decrease slightly in the presence of rifampin, although not significantly.

The metabolite formation clearances are shown in Table III. Disulfiram pretreatment decreased the forma-

Table III. Effect of disulfiram or rifampin on the formation clearance of acetaminophen metabolites

	Formation clearance (mL/h/kg)		
Pathway	*Control*	*Treatment*	*P Value*
Disulfiram			
Acetaminophen glucuronide	232 ± 74.3	211 ± 52.7	.30
Acetaminophen sulfate	115 ± 33.6	107 ± 52.9	.45
Catechol	25.7 ± 8.68	15.5 ± 6.68	.01
Thiols	38.6 ± 23.4	9.92 ± 3.17	.01
Rifampin			
Acetaminophen glucuronide	198 ± 51.6	229 ± 39.4	.55
Acetaminophen sulfate	132 ± 26.9	154 ± 50.4	.09
Catechol	26.2 ± 7.81	33.1 ± 9.95	.09
Thiols	35.7 ± 11.1	39.9 ± 12.3	.43

Data reported are mean values ± SD. Statistical comparisons were made with the Student paired *t* test.

tion clearance values of both proximate oxidative metabolites. The formation clearance of the catechol metabolite was decreased by approximately 40% ($P = .01$) and that of NAPQI was decreased by 74% ($P < .01$). Disulfiram had no effect on the glucuronide or sulfate formation clearances or on the renal clearance of acetaminophen itself. As was the case with urinary metabolite recovery, metabolite formation clearances tended to increase slightly but not significantly after rifampin pretreatment.

DISCUSSION

CYP2E1 contributed markedly to the oxidative metabolism of acetaminophen in humans as shown by (1) the 18% decrease in acetaminophen plasma clearance after pretreatment with the specific and potent CYP2E1 inhibitor disulfiram approximately 10 hours before acetaminophen administration; (2) the reduction in the urinary recovery of the thiol conjugates by 69%, an index of exposure to NAPQI, and the catechol metabolites by 29%; and (3) the inhibition of the formation clearances of NAPQI and the catechol metabolite by 74% and 40%, respectively. Disulfiram had a greater effect on NAPQI than on catechol metabolite formation. In contrast, rifampin caused negligible changes in acetaminophen disposition, suggesting that even when induced, the contribution of CYP3A to NAPQI formation is small at best.

Single-dose disulfiram predominantly inhibits CYP2E1.[14,15,22] Although long-term disulfiram also inhibits CYP1A2, CYP1A2 has been shown not to contribute to NAPQI formation in vivo, as described above[6] and as suggested by in vitro kinetic data.[4] Studies in healthy volunteers have shown that single-dose disulfiram does not significantly inhibit the activity of CYPs 2A6, 2C9, 2C19, 2D6, or 3A4 in vivo.[14,15] Because of the specificity of single-dose disulfiram for CYP2E1 and because of the insignificant contribution of CYP1A2 to NAPQI formation in vivo, the observed decreased NAPQI formation clearance after disulfiram pretreatment suggests that CYP2E1 makes the predominant contribution toward NAPQI formation in humans in vivo.

Selective inactivation of CYP2E1 by single-dose disulfiram pretreatment has been used to establish the predominant role of CYP2E1 in the metabolism of halogenated anesthetics and the formation of toxic metabolites in humans in vivo. Disulfiram inhibited enflurane dehalogenation by 95%[23] and that of sevoflurane and halothane by 76% and 87%, respectively, as assessed by changes in plasma concentrations and urinary excretion of fluoride, bromide, and other metabolites.[24,25] CYP2E1 activity in healthy volunteers, assessed by chlorzoxazone plasma clearance and 6-hydroxychlorzoxazone formation clearance, was inhibited 89% to 95% by single-dose disulfiram.[22,26] These results suggested that at least 85% to 100% of volatile anesthetic metabolism in humans is catalyzed by CYP2E1. In contrast, single-dose disulfiram inhibited NAPQI formation by 69%. Thus it appears that disulfiram inhibited CYP2E1 activity in vivo more completely than it inhibited NAPQI formation clearance in this study. Assuming this is true, it appears that as much as 15% of NAPQI formation clearance is not accounted for by CYP2E1.

Findings of this study support the in vitro human liver microsomal inhibition data that suggest that CYP3A4 is, at most, a minor contributor to NAPQI formation (10%) at therapeutically relevant acetaminophen concentrations.[5] However, they are in conflict with data by Patten et al[4] from expressed human

P450s that suggest that CYP3A4 may contribute as much as 48% to NAPQI formation clearance. Because the reported in vitro K_m for CYP2E1 is much greater than that for CYP3A4, it is not expected that the CYP3A4 contribution would be greater at toxic concentrations of acetaminophen.

The difference between the effects of disulfiram on the formation of the catechol and NAPQI metabolites is consistent with the significantly greater formation of catechol compared with NAPQI by CYP2A6, as found previously in cDNA-expressed enzyme systems.[27] The lack of an effect after disulfiram pretreatment on the urinary clearance of acetaminophen, as well as the formation clearance of acetaminophen glucuronide and sulfate conjugates, suggests that disulfiram altered urinary recovery of acetaminophen and its glucuronide indirectly, that is, through its effects on acetaminophen oxidation.

Paradoxically, rifampin caused a 21% increase in the total clearance of acetaminophen, whereas no differences were detected in the formation clearances of any of the metabolites of acetaminophen. There were trends toward enhanced formation clearances for all metabolites *except* the thiol conjugates.

The clinical application of disulfiram to diminish the toxicity of electrophilic metabolites formed by CYP2E1 from volatile anesthetics has been suggested previously.[25] The present investigation suggests that disulfiram may also be an effective interventional strategy to ameliorate NAPQI-dependent acetaminophen toxification. It therefore appears to be prudent to administer disulfiram to patients in whom acetaminophen overdose is suspected and in whom acetaminophen levels are detectable in plasma. However, the effects of disulfiram on aldehyde dehydrogenase should also be considered. Disulfiram inhibits the elimination of acetaldehyde (the product of ethanol oxidation by alcohol dehydrogenase) by aldehyde dehydrogenase, which accounts for the nausea and vomiting produced when individuals treated with disulfiram consume alcohol. Thus, in individuals who recently have consumed ethanol, the potential detrimental effects of treatment with disulfiram must be kept in mind. In any event, treatment with disulfiram should not replace administration of *N*-acetylcysteine, which augments glutathione stores and thereby protects against toxicity caused through exposure to large amounts of NAPQI.

This study showed that after selective clinical inactivation of CYP2E1, acetaminophen plasma clearance decreased and formation of the putative hepatotoxic metabolite NAPQI was substantially inhibited. No changes in acetaminophen clearance were observed with rifampin treatment. This provides convincing evidence of the predominant participation of CYP2E1 in NAPQI formation in humans in vivo and suggests that the contribution of CYP3A is, at best, minor. Disulfiram may be a potentially useful adjunct to the therapy of patients who have suffered acetaminophen overdose if it is administered while acetaminophen is still present in the body.

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