Cytochrome P4502E1 Inhibition by Propylene Glycol Prevents Acetaminophen (Paracetamol) Hepatotoxicity in Mice without Cytochrome P4501A2 Inhibition

Mikael S. Thomsen1, Steffen Loft1, Dean W. Roberts2 and Henrik E. Poulsen1

1Department of Pharmacology, Panum Institute, University of Copenhagen, Copenhagen, Denmark. 2Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079-9502, U.S.A.

(Received October 27, 1994; Accepted January 12, 1995)

Abstract: Acetaminophen hepatotoxicity is associated with its biotransformation to the reactive metabolite N-acetyl-p-benzoquinone imine that binds to proteins. Two forms of cytochrome P450, CYP2E1 and CYP1A2, have been implicated as primarily responsible for the bioactivation. To determine the relative contributions of these P450's, overnight fasted male NMRI mice were pretreated with 10 ml of 50% v/v propylene glycol/kg or fluvoxamine (10 mg/kg) at ~80 and ~20 min. relative to acetaminophen dosing to inhibit CYP2E1 and CYP1A2, respectively. Mice were sacrificed at 0.5 or 4 hr after a hepatotoxic dose of acetaminophen (300 mg/kg). Propylene glycol or propylene glycol plus fluvoxamine, but not fluvoxamine alone protected against acetaminophen hepatotoxicity as indicated by abolished increase in serum alanine aminotransferase activity, less depletion of hepatic glutathione and lower liver/body weight ratios. Propylene glycol inhibited the activity of CYP2E1 as indicated by 84% reduction in the clearance of 3 mg/kg dose of chloroxazone, whereas fluvoxamine inhibited the activity of CYP1A2 as indicated by 40% reduction in the clearance of a 10 mg/kg dose of caffeine. For this animal model, the data are consistent with the notion that hepatotoxicity is associated with bioactivation of acetaminophen by CYP2E1 but not by CYP1A2.

Acetaminophen, a widely used over-the-counter analgesic, is known to cause potentially lethal hepatic necrosis in humans and animals (Prescott et al. 1971). The toxicity is related to the formation of a toxic metabolite by hepatic cytochrome P450-mediated oxidation (Jollow et al. 1973; Mitchell et al. 1973a; b; Potter et al. 1973; Corcoran et al. 1980). The metabolite has been identified as N-acetyl-p-benzoquinone imine (NAPQI) (Dahlin et al. 1984). After low acetaminophen doses, NAPQI is inactivated by conjugation to glutathione (Mitchell et al. 1973b & 1974). However, following an overdose of acetaminophen, hepatic glutathione is depleted (Mitchell et al. 1973b) and NAPQI binds to hepatic proteins, which is believed to initiate the development of hepatic necrosis (Jollow et al. 1973; Potter et al. 1973; Hinson 1980; Black 1984).

In human liver microsomes, two cytochrome P450 enzymes, cytochrome P4502E1 (CYP2E1) and cytochrome P4501A2 (CYP1A2), have been shown to be principal catalysts of acetaminophen activation (Raucy et al. 1989). Recently, it has also been shown that cytochrome P4503A4 (CYP3A4) in human liver microsomes, activates acetaminophen to the reactive metabolite, NAPQI (Thummel et al. 1993; Patten et al. 1993). Propylene glycol, an in vitro CYP2E1 inhibitor (Snawder et al. 1993), protects against acetaminophen hepatotoxicity in mice (Nelson 1981; Hughes et al. 1991) however, the contribution of CYP1A2 in acetaminophen toxicity has not been investigated in vivo. Fluvoxamine, a new antidepressant and selective serotonin reuptake inhibitor, exhibits potent inhibition of CYP1A2 in human liver microsomes (Brasen et al. 1993).

The present study investigated the contributing roles of CYP2E1 and CYP1A2 to acetaminophen hepatotoxicity in vivo. To this end mice were overdosed with acetaminophen after pretreatment with saline (controls), propylene glycol, and/or fluvoxamine in order to inhibit CYP2E1 and CYP1A2, respectively. The inhibition of these enzymes under these experimental conditions was demonstrated in parallel pharmacokinetic experiments with chloroxazone, a CYP2E1 substrate (Peter et al. 1990) and caffeine, a CYP1A2 substrate, in which CYP1A2 is primarily responsible for the demethylation (Butler et al. 1989; Kalow & Tang 1991; Fuhr et al. 1992).

Materials and Methods

Chemicals. Acetaminophen (Sterling-Winthrop A/S, Copenhagen, Denmark), chloroxazone (kindly donated by Astra AB, Södertälje, Sweden), fluvoxamine (kindly donated by Duphar B.V., Weesp, The Netherlands) and caffeine (Sigma Chemical Co., St. Louis, Mo.) were obtained from the sources indicated. All other chemicals were of commercial grade.

Animals. Male NMRI mice (23–25 g) were fed ad libitum and maintained on a 12 hr light/dark cycle and at a constant temperature of 22°. One-two min. prior to obtaining heparinized blood samples from the axillary artery and sacrifice by cervical dislocation, they were anesthetized by an intraperitoneal injection of a 1:1 solution of Midazolam (Dormicum®, Roche, Basel, Switzerland) and Fen-
ACETAMINOPHEN HEPATOTOXICITY INHIBITION BY PROPYLENE GLYCOL

Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>versus</th>
<th>Fluvoxamine</th>
<th>Water</th>
<th>versus</th>
<th>Propylene glycol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroxazone</td>
<td>133.2±23.44</td>
<td>72.23±24.11</td>
<td>107.3±18.64</td>
<td>17.20±0.801*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8)</td>
<td>(7)</td>
<td>(5)</td>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>11.89±1.23</td>
<td>7.26±0.72*</td>
<td>7.70±0.50</td>
<td>5.24±0.58*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10)</td>
<td>(10)</td>
<td>(14)</td>
<td>(13)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are group estimates±S.E. The number of animals used in the estimation (n), versus indicates a parallel experiment; * P≤0.05.

injected on a Beckman 5 μm ODS column eluted with 25% acetonitrile in 125 μM phosphate buffer at a rate of 1 ml/min. The effluent was monitored by an ESA Coulochem (CA) electrochemical detector with a 5010 cell set at 300 mV for electrode 1 and 790 mV for electrode 2. The intra- and interassay coefficients of variation were less than 10%, respectively.

The clearance was calculated on a graph basis as the product of the elimination rate constant and the volume of distribution, determined by non-linear regression of the log plasma-concentration time curve (each point originating from one animal), using MK-Model mathematical software (Elsevier Biosoft, Cambridge, UK). The half-life was calculated from the elimination rate constant.

Toxicity studies. Groups of 12 mice were pretreated, exactly as described above, with fluvoxamine, propylene glycol, both fluvoxamine and propylene glycol, or vehicle and then dosed with 300 mg/kg acetaminophen by an intraperitoneal injection of 500 μl of 15 mg/ml acetaminophen dissolved in 40° physiologic saline. Four hr after dosing, mice were anaesthetized, bled, and killed as described above, and body and liver weights were recorded. Plasma acetaminophen concentrations were determined by HPLC (Loft et al. 1991). Plasma alanine aminotransferase was measured using a commercial kit (Boehringer Mannheim, Mannheim, Germany) on a Shimadzu UV-2100 UV-visible recording spectrophotometer at 340 nm. In a separate experiment, four groups of four mice each were pretreated as above and killed 30 min. after 300 mg/kg acetaminophen for determination of hepatic glutathione. Glutathione levels were determined by HPLC after derivatisation with monobromobimane (Boesgaard et al. 1995). All acetaminophen-dosed animals were fasted for 18 hr prior to acetaminophen dosing.

Results

Pharmacokinetics studies. In fluvoxamine-pretreated mice the clearance and half-life of caffeine were 0.6 and 1.6 times of control (fig. 1, table 1 and 2). In propylene glycol-pretreated mice, the clearance of caffeine was 0.7 times control, whereas the half-life was 1.6-times of control (fig. 1, table 1 and 2).

After propylene glycol treatment, the clearance and half-life of chloroxazone were 0.16 and 5 times control (fig. 1, table 1 and 2), respectively. After fluvoxamine pretreatment, the clearance of chloroxazone was 0.54 times of control and the half-life was similar to the corresponding control.

Toxicity studies. In control mice acetaminophen overdose caused a 20-fold increase in plasma alanine aminotransferase, whereas propylene glycol pretreatment completely abolished this increase. Propylene glycol also protected against the depletion of hepatic glutathione by acetaminophen (table 3). Flavoxamine showed no significant protection against alanine aminotransferase increase or glutathione depletion. The combination of flavoxamine and propylene glycol showed the same protective effect as propylene glycol alone. Acetaminophen concentrations 4 hr after overdose were not significantly different among the four groups.

Discussion

In the present study pretreatment of mice with propylene glycol abolished acetaminophen hepatotoxicity as well as inhibited chloroxazone metabolism, a marker of CYP2E1 activity, whereas fluvoxamine pretreatment had no effect on

Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>versus</th>
<th>Fluvoxamine</th>
<th>Water</th>
<th>versus</th>
<th>Propylene glycol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroxazone</td>
<td>2.26±0.59</td>
<td>2.35±1.23</td>
<td>3.59±1.11</td>
<td>17.30±1.15*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8)</td>
<td>(7)</td>
<td>(5)</td>
<td>(7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>55.17±10.33</td>
<td>87.67±11.01*</td>
<td>62.46±6.97</td>
<td>97.69±14.75*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10)</td>
<td>(10)</td>
<td>(14)</td>
<td>(13)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are group estimates±S.E. The number of animals used in the estimation (n), versus indicates a parallel experiment; * P≤0.05.
Hepatoprotective effects of fluvoxamine and propylene glycol in acetaminophen-overdosed mice.

<table>
<thead>
<tr>
<th>Pretreatment Treatment</th>
<th>No saline</th>
<th>No acetaminophen</th>
<th>Fluvoxamine + acetaminophen</th>
<th>Propylene glycol + acetaminophen</th>
<th>Propylene glycol + fluvoxamine + acetaminophen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (IU/L)</td>
<td>79±72 (6)</td>
<td>1281±765* (11)</td>
<td>1763±1266* (11)</td>
<td>55±39 (12)</td>
<td>49±23 (12)</td>
</tr>
<tr>
<td>Liver/body weight (&lt;100)</td>
<td>5.35±0.36 (6)</td>
<td>6.46±0.72* (11)</td>
<td>6.56±0.75* (12)</td>
<td>5.75±0.22* (12)</td>
<td>6.08±0.35* (12)</td>
</tr>
<tr>
<td>Hepatic GSH (μmol/g)</td>
<td>4.69±0.63 (4)</td>
<td>0.59±0.19* (4)</td>
<td>0.95±0.45* (4)</td>
<td>3.46±0.52** (4)</td>
<td>4.40±0.79* (4)</td>
</tr>
<tr>
<td>Plasma acetaminophen (μg/ml)</td>
<td>not detected</td>
<td>44.48±57.48 (11)</td>
<td>80.27±122 (12)</td>
<td>51.47±25.8 (12)</td>
<td>118.5±92.2 (12)</td>
</tr>
</tbody>
</table>

1 Overnight fasted male NMRI mice were dosed with 300 mg/kg acetaminophen or saline and samples obtained after 4 hr or 30 min. (GSH). Mean±S.D. sample size (n).

* P≤0.05 versus No pretreatment + Saline treatment; † P≤0.05 versus (No pretreatment and Fluvoxamine pretreatment) + Acetaminophen treatment.

acetaminophen toxicity although it reduced the clearance of caffeine, a marker of CYP1A2 activity. Accordingly, CYP2E1 appears to be the main CYP responsible for acetaminophen activation in this animal model.

Our results confirm reports (Hughes et al. 1991; McRae et al. 1991) that the hepatotoxicity of acetaminophen is abolished or reduced if propylene glycol is administered concurrently with, or one hour before acetaminophen administration. This is consistent with the hypothesis that propylene glycol acts as a competitive inhibitor of acetaminophen bioactivation and with the protective effect of a constant dose of propylene glycol being saturable by higher doses of acetaminophen (Snawder et al. 1993). This finding explains the very high doses of acetaminophen required to achieve hepatotoxicity in mouse models when propylene glycol is used as a solvent for toxicology studies (Bartolone et al. 1987; 1988; Placce et al. 1987). The propylene glycol-induced reduction of chlorozoxazone clearance to 16% of control values, as well as in vitro data (Snawder et al. 1993) indicates that propylene glycol is an inhibitor of mouse CYP2E1.

The propylene glycol dose used in the present study (260 mg/kg body weight administrated orally) may inhibit other CYPs than 2E1. However, under conditions that nearly abolished CYP2E1 activity, the clearance of caffeine was reduced to 0.7-times control indicating minimal inhibition of CYP1A2 or other CYPs involved in caffeine elimination. In vitro, propylene glycol in concentrations of 25 mM did not inhibit the phenacetin-O-deethylation activity (Snawder et al. 1993), which is catalysed predominantly by CYP1A2 (Guengerich 1989). Nevertheless, possible inhibition of other CYPs by propylene glycol has yet to be investigated. Particularly the influence of CYP3A4 is interesting, because it may take part in human liver metabolism of acetaminophen (Thummel et al. 1993; Patten et al. 1993). CYP2E1 accounts for approximately 70% of the acetaminophen bioactivation in human liver microsomes (Rauch et al. 1989). In mice, the apparently selective inhibition of chlorozoxazone clearance and acetaminophen toxicity by propylene glycol indicates that CYP2E1 is the dominant CYP form activating acetaminophen, and suggests that the contribution of other CYP forms to activation of acetaminophen in the liver is negligible.

Difficulties in interpreting the contributions of CYP2E1 and CYP1A2 to acetaminophen toxicity may relate to specific kinetic circumstances due to differences in the Michaelis constant, K_m, and maximal velocity, V_max (Snawder et al. 1994). At very high acetaminophen concentrations, CYP1A2 with low affinity and high velocity may play a significant role which is not apparent at lower concentrations. In other organs, e.g. in the kidney, there might be another distribution of the enzymes. In evaluating an inhibitor for clinical use, a potent and non-specific inhibitor of several CYPs might be preferable to a specific inhibitor that might work only in one organ.

Fluvoxamine is a potent inhibitor of CYP1A2 with a K_i of 0.4 μM in human liver microsomes (Brosen et al. 1993). However, in the present study fluvoxamine reduced the clearance of caffeine, a traditional marker of CYP1A2 activity (Kalow & Tang 1991), by only 40%. This may be due to a lack of potency of fluvoxamine with respect to mouse CYP1A2. In addition other CYPs than 1A2 that are not inhibited by fluvoxamine may contribute significantly to caffeine elimination in mice (Berthou et al. 1992). Fluvoxamine reduced the clearance of chlorozoxazone by 45% although the half-life was unchanged. However, in human liver microsomes fluvoxamine is a specific CYP2A1 inhibitor without effects on CYP2E1 and CYP3A4 (unpublished observation). The metabolism of several other drugs is inhibited by fluvoxamine in humans in vivo (Brosen et al. 1993), and more detailed elucidation of its inhibitory specificity is warranted.

An additional protective effect of propylene glycol against acetaminophen toxicity could relate to its metabolism which produces NADH. NADH inhibits the citric acid cycle and depletes intermediates such as malate and α-
ACETAMINOPHEN HEPATOTOXICITY INHIBITION BY PROPYLENE GLYCOL

Ketoglutarate are necessary for the generation of cytoplasmic NADPH via shuttle mechanisms. Rates of oxidation by cytochrome P450 forms in intact cells are limited by the generation of NADPH. This hypothesis is proposed for the function of ethanol on acetaminophen toxicity (Thurman et al. 1977; Reineke et al. 1980).

In conclusion, in mice the protective effect of propylene glycol against acetaminophen hepatotoxicity appears related to a specific inhibition of CYP2E1. The approach used in the present study may be used for inspection of the relative importance of CYP enzymes in man in vivo.

References


