Acetaminophen Metabolism Does Not Contribute to Gender Difference in Its Hepatotoxicity in Mouse

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Gender is an important factor in pharmacokinetics and pharmacodynamics. In the current study, gender difference in acetaminophen (APAP)-induced hepatotoxicity has been examined. Male and female mice were injected with a toxic dose of APAP (500 mg/kg, ip). Female mice were resistant to the hepatotoxic effects of APAP, depicted by serum alanine aminotransferase and sorbitol dehydrogenase activities and histological analysis. Basal hepatic reduced glutathione (GSH) levels were lower in females than in males, suggesting that basal GSH level may not be a factor in determining the gender difference of APAP hepatotoxicity. APAP metabolism was slower in females than males, revealed by lower levels of glucuronidation and sulfation and higher amounts of free APAP in the livers of female mice. Lower basal Cyp1a2 mRNA levels and lower expression of Cyp1a2 and Cyp3a11 mRNAs after APAP dosing were also observed in females compared with males. However, there was no gender difference in N-acetyl-p-benzoquinone imine covalent binding 2 h after APAP administration, suggesting similar APAP bioactivation between genders. Moreover, liver Gst pi mRNA levels were significantly lower in females than males. This finding is consistent with a previous report, which showed that Gst pi knockout mice are protected from APAP-induced liver toxicity. In conclusion, gender difference of APAP-induced hepatotoxicity is not likely due to APAP metabolism. Perhaps, it is in part due to gender-dependent Gst pi expression. However, the mechanism underlying the association between reduction in Gst pi expression and hepatoprotective effect against APAP toxicity remains to be further explored.

Key Words: acetaminophen; gender; hepatotoxicity; cytochrome P450; glutathione S-transferase; glutathione.

Gender is an important factor that contributes to differences in pharmacokinetics, including drug absorption, distribution, uptake, metabolism, and excretion, and pharmacodynamics, including drug effects and toxicity (Morris et al., 2003; Tanaka and Hisawa, 1999). Gender differences in drug metabolism and elimination are mainly related to steroid hormone levels, in addition to physical constitution (body water space, muscle mass, organ blood flow, and organ function) and physiology (menopause, pregnancy, and menstruation) (Tanaka and Hisawa, 1999). Differential gene expression and activities of drug-metabolizing enzymes are the fundamental mechanisms of gender difference in drug metabolism. Clinically, drug side effects and toxicity arising from sex differences must be considered.

Acetaminophen (APAP) is a widely used analgesic and antipyretic. At therapeutic doses, the majority of APAP is conjugated with sulfate or glucuronide and then excreted into bile and urine, whereas a small portion of APAP is oxidized by cytochrome P450s (Cyps) like Cyp1a2, Cyp3a11, and Cyp2e1 to a toxic electrophile N-acetyl-p-benzoquinone imine (NAPQI) (Dahlin et al., 1984). NAPQI is inactivated by conjugation with reduced glutathione (GSH) to form a 3-S-glutathionyl conjugate of APAP (APAP-GSH). At toxic doses, sulfation and glucuronidation pathways become saturated, causing more NAPQI to be formed along with the rapid depletion of hepatic GSH. Accumulated NAPQI covalently binds to cellular macromolecules (Jollow et al., 1973; Potter et al., 1973) and also causes production of reactive oxygen species, leading to oxidative stress (Lores Arnaiz et al., 1995). Overdose of APAP causes severe liver injury and acute liver failure (Davidson and Eastham, 1966; Mitchell, 1988).

Despite a massive experimental literature related to APAP, gender difference of APAP-induced liver toxicity has not been studied intensively. The present study aims at exploring the molecular mechanisms underlying gender difference in APAP-induced hepatotoxicity. We examined APAP-induced liver injury, GSH levels, gene expression of enzymes involved in APAP metabolism, and APAP metabolites in male and female mice.

MATERIALS AND METHODS

Reagents. Standards of APAP metabolites, e.g., APAP-glutathione (APAP-GSH), APAP-cysteinylglycine (APAP-CG), APAP-cysteine (APAP-CYS), APAP-mercapturate (APAP-NAC), APAP-glucuronide (APAP-GLU), and APAP-sulfate (APAP-SUL), were generous gifts from Dr Curtis Klaassen.
(University of Kansas) and Dr Jose Manautou (University of Connecticut). APAP, GSH, and EDTA were purchased from Sigma-Aldrich (St Louis, MO). High-performance liquid chromatography (HPLC)–grade methanol and acetic acid were obtained from Fisher Scientific (Springfield, NJ).

**Mice.** Age-matched mice with a background of C57/B6 and 129/Sv were kept (3 mice per cage) in steel microisolator cages at 22°C with a 12/12-h light/dark cycle. Food and water were provided ad libitum throughout the entire feeding period. Female and male mice (10–12 weeks old) were fasted overnight and then treated with APAP (500 mg/kg, ip) or vehicle (phosphate-buffered saline) for 1, 2, 6, or 18 h. Mice were fasted because unfed mice exhibit more consistent responses to APAP than do fed mice (Lucas et al., 2000; Placke et al., 1987). Mice were sacrificed at the indicated time points. Bile and urine were collected from gallbladders and bladders at 1 h after APAP treatment to measure APAP metabolites, considering fast metabolism of APAP. At 2 and 18 h after APAP administration, blood samples were collected for alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) analyses to monitor the liver injury at early and late stages of APAP overdose, respectively. At the same time, liver samples were also fixed in 10% formalin and stained with hematoxylin and eosin for histological visualization of the cellular damage at early and late stages of APAP toxicity. At 2 h after APAP dosing, mouse livers were collected for preparing total RNA and tissue homogenate to measure mRNA expression of phase I genes involved in APAP metabolism and GSH content. At 6 h after APAP treatment, liver samples were collected for total RNA preparation to access the induction of antioxidant proteins. All the animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

**ALT and SDH assays.** Plasma ALT and SDH activities were determined spectrophotometrically using assay reagents from Catachem Inc. (Bridgeport, CT), according to the manufacturer’s instructions. Briefly, 20 μl of serum was added to 0.5 ml of reagents. The samples were incubated at 37°C for 1 h and then assayed for the decrease in absorbance at 340 nm. The activities of ALT and SDH were expressed as the number of international units per liter based on the following equation: ALT or SDH units/l = [absorbance change (A)/min × assay volume (ml) × 1000]/[6.22 × light path (cm) × sample volume (ml)] = A/min × 2572.

**Measurement of GSH levels.** GSH level was determined by the recycling method (Tietze, 1969).

**HPLC analysis.** APAP and its metabolites in the liver, blood, bile, and urine were analyzed using an HPLC method described by Chen et al. (2003) and Lucas et al. (2000) with minor modification. Briefly, livers (0.1 g) were homogenized in 9 volumes of ice-cold methanol. Perchloric acid (3 N) was added to 2 volumes of serum samples and liver homogenates to precipitate proteins. Bile and urine samples were diluted with 2 volumes of ice-cold HPLC-grade methanol. After centrifugation, supernatants were filtered through a 0.2-μm nylon filter and used for analysis. Aliquots (20 μl) of the processed samples were injected into a Vydac 208TP54 5-μm C-8 column (4.6 × 250 mm) (P. J. Cobert Associates, St Louis, MO). APAP and its metabolites were separated with a linear gradient and a constant flow rate of 1 ml/min. Solvent A consisted of 1% aqueous acetic acid; solvent B was 100% methanol. The mobile phase was initially kept at 5% B for 4 min followed by a 1-min linear gradient that finished at 12.5% B, and then 20 min at 12.5% B. The elution of metabolites was monitored at 254 nm. Retention times of APAP and its metabolites were determined using authentic standards. Since the retention times of APAP-CG and APAP-CYS were too close to separate, they were quantified together as APAP-CG/CYS. Samples from the vehicle-treated mice showed no interfering peaks. Quantification was based on integrated peak areas. The concentrations of APAP and its metabolites were calculated using an APAP standard curve.

**Covalent binding analysis of 14C-APAP.** Total covalent binding of 14C-APAP is used to estimate NAPQI production, which reaches a peak 2 h after APAP treatment (Jollow et al., 1973). Male and female mice were treated with 14C-APAP (500 mg/kg, ip, 4.3 μCi/mmol, 4 μCi per mouse. Sigma Chemical Co.). Two hours after dosing, livers were collected and flash frozen. The procedure to analyze the 14C-APAP covalently bound to liver macromolecules was described by Jollow et al. (1973). Briefly, liver tissue (200 mg) was homogenized in 1 ml of 0.9% saline. Total protein was precipitated in 2 ml of trichloroacetic acid (TCA, 0.9M), and then centrifuged at 1000 × g for 15 min at room temperature. After removing the supernatant, protein precipitate was resuspended in 3 ml of TCA (0.6M), vortexed, and centrifuged at 1000 × g for 3 min. This washing step was repeated two more times. Then, the pellet was washed six times with 80% methanol (3 ml per wash), and then dissolved in 2 ml of NaOH (1M). An aliquot of the dissolved pellet was used to measure 14C using a liquid scintillation counter (Packard Instrument Co., Meriden, CT).

**Northern blot analysis.** Total liver RNA was extracted by a guanidinium isothiocyanate method (Chomczynski and Sacchi, 1987). RNA concentration was determined spectrophotometrically. Twenty micrograms of total RNA was separated by electrophoresis in a denaturing 1.2% (wt/vol) agarose gel containing 2.2M formaldehyde. Equal loading per lane was assessed by ethidium bromide staining and hybridization with a β-actin cDNA probe. RNA was transferred to a nylon membrane by capillary blotting in 10× standard saline citrate and cross-linked by UV irradiation. Probe labeling and hybridization were performed as described previously (Wan et al., 2003). The differences in mRNA levels were determined by densitometry, normalized to β-actin mRNA levels, and expressed as fold difference compared with controls.

**Statistical analysis.** Data were given as mean ± SD. Statistical analysis was performed using the Student t-test or one-way analysis of variance. Significance was defined by p < 0.05.

**RESULTS**

**Modulation of APAP-Induced Hepatotoxicity by Gender**

To evaluate the effect of gender on hepatocyte susceptibility to APAP toxicity, both male and female mice were treated with APAP (500 mg/kg, ip) or vehicle and sacrificed 2 or 18 h after dosing. Liver injury was evaluated histologically and by measuring serum ALT and SDH levels. Two hours after APAP treatment, levels of serum ALT and SDH were not significantly changed in either males or females (Fig. 1). Histopathological analysis revealed that modest cellular damage occurred in the livers of male mice, whereas only minor cellular damage was seen in the livers of female mice (Fig. 2). Eighteen hours after APAP injection, serum ALT and SDH levels were 14-fold and 7-fold higher in males than in females, respectively (Fig. 1). Consistent with this observation, massive centrilobular necrosis was present in the livers of males, but not females (Fig. 2). Vehicle-treated mice displayed normal liver histology (data not shown). These data indicate that, in the strain of C57/B6-129/Sv mice, gender modulates susceptibility to hepatotoxicity caused by APAP. While male mice are susceptible, female mice are highly resistant to the hepatotoxicity caused by APAP overdose.

**Rapid Depletion of Hepatic GSH in Both Genders after APAP Treatment**

GSH level is critical for the detoxification of NAPQI. Total GSH content was assayed using livers harvested from mice 2 h after vehicle or APAP administration. Surprisingly, the basal
hepatic GSH levels were significantly lower in females than in males (Fig. 3). After APAP treatment, both male and female mice showed a similar rapid depletion of hepatic GSH (Fig. 3). These data suggest that basal GSH level may not contribute to the gender difference in susceptibility to APAP-induced hepatotoxicity.

**Slower Metabolism of APAP in Females Compared with Males**

APAP is metabolized through several pathways. To elucidate the mechanism underlying the protection of female mice from APAP-induced hepatotoxicity, APAP metabolites were measured. Liver, serum, urine, and bile samples were collected from both male and female mice 1 h after APAP administration. HPLC analyses of free APAP and APAP metabolites were performed. The results were summarized in Table 1.

There were no significant differences in the concentrations of free APAP between male and female mice in bile, urine, and serum, which may suggest that absorption, distribution, uptake, and excretion of free APAP are similar between genders. However, significantly higher concentrations of free APAP were observed in female livers. This finding depicts a slower rate of metabolism of APAP in female versus male livers. The majority of APAP is metabolized through sulfation and glucuronidation pathways and then excreted mainly into urine and bile (Chen et al., 2000a). We found that the concentrations of APAP-SUL and APAP-GLU in urine were significantly lower in female than in male mice. The data indicate slower sulfation and glucuronidation of APAP in female mice, which results in greater accumulation of free APAP in the livers of females than males. Oxidation of a small portion of APAP by Cyp enzymes gives rise to NAPQI, which is inactivated by conjugation with GSH. APAP-GSH is mostly excreted into bile (Chen et al., 2003). At the same time, a portion of the APAP-GSH is sequentially converted to APAP-CG, APAP-CYS, and APAP-NAC, which are excreted into urine and bile (Chen et al., 2003). Comparing the concentrations of APAP-GSH and sequential APAP conjugates in the liver, bile, urine, and serum showed no significant differences between genders. This observation suggests that GSH-dependent NAPQI detoxification efficiency may not be gender related.
Gender Differences in the Expression of Phase I Genes Involved in APAP Metabolism

Three Cyp enzymes (Cyp1a2, Cyp3a11, and Cyp2e1) are known to oxidize APAP to toxic NAPQI in mice (Guo et al., 2004; Zaher et al., 1998). Levels of these Cyp enzymes control the amount of NAPQI formation. To determine if there are gender differences in the expression of these enzymes at the mRNA level, northern blot hybridizations were performed with total RNA extracted from liver samples collected 2 h after APAP or vehicle treatment. The results are shown in Figure 4. Comparing basal mRNA levels, Cyp1a2 mRNA expression was significantly lower in females than males, whereas no differences were observed for Cyp3a11 and Cyp2e1 between genders. After APAP administration, the expression of Cyp1a2, Cyp2e1, and Cyp3a11 was significantly reduced in females. The situation was the same in males except that the expression of Cyp3a11 mRNA, which although reduced, was not statistically significant. Comparing mRNA levels after APAP injection, Cyp1a2 and Cyp3a11 expression was lower in females than in males. APAP treatment decreased the expression of Cyp2e1 to a similar extent in both genders. To examine whether the gender differences of phase I gene expression result in differential APAP bioactivation, the following covalent binding analysis of APAP was performed.

Covalent Binding Analysis of 14C-APAP

Measurement of total covalent binding of 14C-APAP is a reliable assessment of NAPQI production, which peaks 2 h after APAP treatment in mice (Jollow et al. 1973). Therefore, 2 h after dosing with 14C-APAP, livers were collected and covalent binding analysis of 14C-APAP was performed. The results showed that there was no significant gender difference in NAPQI covalent binding (Fig. 5), suggesting similar bioactivation of APAP between genders.

Gender Differences in the Expression of Phase II Genes Involved in APAP Metabolism

Gsts participate in catalyzing the conjugation of NAPQI with GSH and represent a superfamily composed of at least 20 members in mice (Hayes et al., 2005). Gst pi was shown to efficiently use NAPQI as a substrate in vitro (Coles et al., 1988). However, it is unknown which member preferentially or specifically catalyzes NAPQI-GSH conjugation in vivo. Hence, we measured the hepatic gene expression profile of the Gst family members. Liver total RNA was extracted from male and female mice treated with vehicle or APAP for 2 h. Northern blot experiments were performed with specific cDNA probes synthesized by reverse transcriptase–polymerase chain reaction (Fig. 6). Because of a greater than 95% sequence identity between Gst alphal and 2 or Gst mu1 and 3, a common probe Gst alphal/2, detecting both Gst alphal and 2 mRNAs, and another common probe Gst mu1/3, detecting both Gst mu1 and 3 mRNAs, were prepared. The results revealed a gender-dependent gene expression profile for the Gst family. The basal mRNA levels of some Gsts (Gst alphal/2, alphal3, mu2, mu4, and omega1) were comparable between genders, but gene expression for the majority of members was higher in the livers of males than those of females. In particular, gene expression levels for three Gsts (Gst pi1, mu3v, and zeta1) were 10-fold higher in males than those of females.

Table 1

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Note. Animals were sacrificed 1 h after APAP injection (500 mg/kg, ip). Liver, bile, urine, and blood samples were collected and prepared for HPLC analyses of APAP and its metabolites. Note that lower glucuronidation, lower sulfation, and higher hepatic free-APAP concentration indicated slower APAP metabolism in females than in males. The data are presented as mean ± SD (n = 5). ND, not detectable.

*p < 0.05;
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FIG. 3. Hepatic GSH levels in vehicle- (C) or APAP-treated mice. Liver samples were collected 2 h after vehicle or APAP treatments. GSH content was measured. Note that the basal level of GSH was lower in female mice than in male mice (p < 0.05). Hepatic GSH was rapidly depleted in both genders after APAP administration. The data are presented as mean ± SD (n = 5).

FIG. 4. Liver total RNA was extracted from male and female mice treated with vehicle or APAP for 2 h. Northern blot experiments were performed with specific cDNA probes synthesized by reverse transcriptase–polymerase chain reaction (Fig. 6). Because of a greater than 95% sequence identity between Gst alphal and 2 or Gst mu1 and 3, a common probe Gst alphal/2, detecting both Gst alphal and 2 mRNAs, and another common probe Gst mu1/3, detecting both Gst mu1 and 3 mRNAs, were prepared. The results revealed a gender-dependent gene expression profile for the Gst family. The basal mRNA levels of some Gsts (Gst alphal/2, alphal3, mu2, mu4, and omega1) were comparable between genders, but gene expression for the majority of members was higher in the livers of males than those of females. In particular, gene expression levels for three Gsts (Gst pi1, mu3v, and zeta1) were 10-fold higher in males than those of females.

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higher in males compared with females. These findings clearly demonstrate gender-dependent gene expression patterns of Gst family members in the liver. In both genders, APAP treatment either inhibited or did not appreciably alter the expression of Gst genes.

Gender Differences in the Expression of Heme Oxygenase 1 and Antioxidant Protein 2 Genes

Oxidative stress caused by NAPQI is an alternative mechanism of APAP-induced hepatotoxicity (Jollow et al., 1974). Heme oxygenase 1 (HO-1) and antioxidant protein 2 (AOP-2) are two important antioxidant proteins, whose expression is associated with Gst pi level in male mice (Elsby et al., 2003; Kitteringham et al., 2003). It was suggested that elevated expression of the HO-1 and AOP-2 genes may protect mice from APAP-induced liver toxicity (Elsby et al., 2003; Kitteringham et al., 2003). Evidence showed that HO-1 gene expression was induced 6 h after APAP treatment in male mouse liver (Aleksunes et al., 2005). Therefore, the mRNA expression of HO-1 and AOP-2 in the liver was measured in male and female mice treated with vehicle or APAP for 6 h. The significant gender difference of HO-1 expression is shown in Figure 7. The data showed that the hepatic basal level of HO-1 mRNA expression was 20-fold higher in males than in females. APAP treatment significantly induced the expression of HO-1 genes in both males and females. The result suggests that HO-1 might not be the factor determining the gender difference of...
APAP-induced hepatotoxicity, since higher basal expression levels of HO-1 gene in male mouse livers are not expected to be associated with greater susceptibility to NAPQI-induced oxidative stress. There was no significant gender difference of AOP-2 gene expression (Fig. 7). In addition, APAP treatment did not influence the expression of AOP-2 gene in both genders. Therefore, AOP-2 may not contribute to the gender difference in APAP hepatotoxicity.

**DISCUSSION**

The present study, using the mouse strain with a background of C57/B6 and 129/Sv, demonstrates that gender modulates APAP-induced hepatotoxicity. Female mice are resistant, whereas male mice are much more susceptible, to the hepatic toxic effect of APAP overdose. Chan et al. (2001) observed that the survival rate of female mice (C57/SV129) was about 6-fold higher than that of male mice 48 h after APAP administration at a dose of 500 mg/kg. Our finding is consistent with the report. In the rat, lower susceptibility of APAP-induced hepatotoxicity was also observed in young adult, but not in aging, females (Raheja et al., 1983; Tarloff et al., 1996). Some reports, however, showed that no evident gender difference of APAP-induced hepatotoxicity was seen in CD-1 mice (Chen et al., 2000b; Hoivik et al., 1995). The conflicts of literature might be due to the differences in the strains of mice used in these studies. It has been shown that both rodents and humans are very sensitive to the hepatotoxic effects of APAP, developing similar centrilobular necroses (Davis et al., 1974). APAP hepatotoxicity occurs not only due to overdose but also by therapeutic dose in the presence of risk factors such as alcohol consumption, fasting, drug interaction, or preexistence of liver diseases (Sumioka et al., 2004). It is important to determine whether there is a gender difference in APAP hepatotoxicity in humans. GSH plays a pivotal role in drug detoxification. Our results showed a gender difference in the total hepatic GSH levels in

![FIG. 6. Northern blot analysis of Gst gene expression. Mice were sacrificed 2 h after vehicle (C) or APAP treatments. Total liver mRNA was extracted and subjected to northern blot analyses using the indicated probes. The Gst alpha1/2 probe detected both Gst alpha1 and 2 mRNAs. The Gst mu1/3 probe detected both Gst mu1 and 3 mRNAs. The mRNA levels were measured by densitometry, normalized to β-actin mRNA levels, and expressed as relative fold differences by the numbers under each representative hybridization band (n = 5). Note that gene expression profiles of the Gst family were different due to gender or APAP treatment. mGst, microsomal Gst; Gst mu3v, Gst mu3 variant, a new Gst gene that has an mRNA sequence similar to that of Gst mu3 (GenBank ID: XM_137047).](https://academic.oup.com/toxsci/article-abstract/92/1/33/1642954)
between genders. In female mice, liver Cyp1a2 mRNA levels decreased significantly compared with male mice. This difference was demonstrated in various studies, and the mechanism underlying the gender-dependent susceptibility of APAP hepatotoxicity is currently under investigation.

Further analyses revealed a similar bioactivation of APAP between genders. In female mice, liver Cyp1a2 mRNA levels were significantly lower than those in males, which was also the case after APAP treatment. Among the phase I Cyp enzymes, Cyp2e1 is the major protein that bioactivates APAP at a lower dose (150 mg/kg), whereas at a higher dose (400 mg/kg) Cyp1a2 becomes the key enzyme for APAP bioactivation (Snawder et al., 1994). Cyp3a11 is also critical for NAPQI formation (Guo et al., 2004). Our data illustrate differential Cyp3a11 gene expression in response to APAP treatment between males and females. APAP administration significantly decreased the expression of Cyp3a11 mRNA in females, but not in males. However, our study revealed that NAPQI production is similar between genders, indicating that APAP bioactivation does not contribute to the mechanisms underlying the gender-dependent susceptibility of APAP hepatotoxicity.

The gender difference in the gene expression profile of the Gst family was revealed in the current study. Of particular interest is the gender-dependent expression pattern of Gst pi. Androgen regulates Gst pi expression (Ikeda et al., 2002), which may explain the gender difference in the expression level of the gene. Interestingly, Park’s group demonstrated that Gst pi knockout male mice are resistant to APAP-induced hepatotoxicity (Henderson et al., 2000). In line with the close correlation between Gst pi and susceptibility to APAP hepatic toxicity, we found that female mice are resistant to APAP hepatic toxicity and have 10-fold less liver Gst pi mRNA compared with male mice. It has been shown that Gst pi is efficient in catalyzing APAP-GSH conjugation in vitro (Coles et al., 1988). However, it may not contribute to the formation of APAP-GSH conjugate in vivo (Henderson et al., 2000).

Although a similar rapid depletion of GSH was found in both wild-type and Gst pi knockout mice, GSH levels only recovered in the Gst pi–null mice (Henderson et al., 2000). This finding indicated that Gst pi may play a role in maintaining GSH homeostasis in response to oxidative stress. Evidence indicated that Gst pi likely functions as a direct inhibitor of c-Jun N-terminal kinase (JNK) and regulates the constitutive expression of specific downstream molecular targets of the JNK signaling pathway, such as HO-1 (Elsby et al., 2003). The lack of Gst pi elevates the expression levels of HO-1 in the liver (Elsby et al., 2003), which may suggest an explanation why Gst pi–null mice are protected from APAP-induced hepatotoxicity. However, our data showed that the hepatic level of HO-1 mRNA is at least 20-fold lower in females than in males. Additionally, Kitteringham et al. (2003) found that AOP-2, a member of the thiol-specific antioxidant protein family, is expressed at a higher level in the liver of Gst pi–null mice. However, we did not find an inverse correlation between the expression of AOP-2 and Gst pi in female mice. As a matter of fact, the AOP-2 mRNA level in female liver is similar to that in male liver. Therefore, HO-1 and AOP-2 cannot account for the resistance of female mice to APAP-induced hepatotoxicity. The gender-dependent role of Gst pi in APAP hepatotoxicity needs to be investigated.
Even though two hepatoprotective pathways (basal liver GSH and HO-1 mRNA expression) are significantly lower in C57/B6-129/Sv female mice, they are still more resistant to APAP hepatotoxicity than their male counterparts. This illustrates the magnitude and/or importance of the presence of an unknown pathway conferring protection to this strain of female mice.

In summary, our study showed that gender modulates APAP-induced hepatotoxicity in mice. Males are highly susceptible, whereas females are strongly resistant, to the hepatic toxic effects of APAP overdose. Gender-dependent APAP metabolism does not contribute to the mechanisms underlying this observation. Gst pi might play an important role in gender-dependent susceptibility of APAP-induced hepatotoxicity. More studies are required to determine why reduced expression of Gst pi in females and genetic deletion of the Gst pi gene are associated with resistance to APAP toxicity.

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