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In Vivo Mechanisms of Tissue-Selective Drug Toxicity: Effects of Liver-Specific Knockout of the NADPH-Cytochrome P450 Reductase Gene on Acetaminophen Toxicity in Kidney, Lung, and Nasal Mucosa

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ABSTRACT

Acetaminophen overdose causes toxicity in liver and extrahepatic tissues. Although it is well established that cytochrome P450 enzymes play a critical role in the metabolic activation of acetaminophen, it is not yet clear whether acetaminophen toxicity in extrahepatic tissues is a consequence of hepatic biotransformation. The aim of this study was to determine whether extrahepatic acetaminophen toxicity is altered in a mouse model that has liver-specific deletion of the NADPH-cytochrome P450 reductase (*Cpr*) gene. Liver-specific *Cpr*-null (Null) mice were resistant to acetaminophen hepatotoxicity, and they showed faster acetaminophen clearance than did wild-type mice at a toxic acetaminophen dose (400 mg/kg i.p.). However, when circulating acetaminophen levels were made equivalent in the two strains, the severity of extrahepatic acetaminophen toxicity was decreased in the Null relative to that in the wild-type mice in the lung, kidney, and lateral nasal glands, although not in the nasal olfactory and respiratory mucosa. In the lung and liver, the decreased acetaminophen toxicity was accompanied by substantial decreases in the formation of acetaminophen-protein adducts in the Null mice; adducts were not detected in other tissues examined. These results indicate that acetaminophen toxicity in the nasal mucosa is not dependent on hepatic microsomal P450-catalyzed metabolic activation and that acetaminophen toxicity in the lung, kidney, and lateral nasal glands is at least partly caused by liver-derived acetaminophen metabolites.

An important issue in the mechanisms of xenobiotic metabolism and chemical toxicity is whether cytochrome P450 (P450) enzymes expressed in various extrahepatic target tissues are critical for the tissue-selective toxicity of chemical compounds. It is well established that liver plays a major role in systemic drug clearance, although bioavailability may be affected by metabolism at the portals of entry (Ding and Kaminsky, 2003). It is also believed, although not proven in most cases, that P450s of extrahepatic target tissues play important roles in metabolic activation. However, reactive as well as nonreactive intermediate metabolites are also generated in the liver, and they may be transported from liver to other sites. Because the total metabolic capacity of the liver is so much greater than the metabolic capacity of an extrahepatic tissue (e.g., the nasal mucosa), it is often difficult to determine the relative contributions of liver and extrahepatic tissues to target-tissue toxicity.

Acetaminophen, a commonly used antipyretic and analgesic agent, is widely used as a model compound for studying the mechanisms of chemical toxicity. Acetaminophen overdose causes liver and kidney damage in some humans (Boyer and Rouff, 1971) and in experimental animals (Boyd and Bereczky, 1966; Mitchell et al., 1973). In mice, acetaminophen also causes toxicity in several other organs, including the nasal mucosa and lung (Placke et al., 1987; Jeffery and Haschek, 1988; Genter et al., 1998). The toxicity of acetaminophen is believed to be associated with P450-mediated generation of a toxic metabolite, *N*-acetyl-*p*-benzoquinone imine (Dahlin et al., 1984), which causes glutathione depletion and

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ABBREVIATIONS: P450, cytochrome P450; CPR, NADPH-cytochrome P450 reductase; OE, olfactory epithelium; GS-AP, acetaminophen-glutathione conjugate; ALT, alanine aminotransferase; LNG, lateral nasal gland; NPSH, nonprotein sulfhydryl.

adduction with cellular macromolecules (Jollow et al., 1973; Potter et al., 1974). In addition, P450-catalyzed acetaminophen metabolism leads to the formation of 3-hydroxy-acetaminophen, a catechol that is hepatotoxic (Forte et al., 1984). The two metabolites may derive from a common precursor, as has been hypothesized previously (Forte et al., 1984; Genter et al., 1998), or they may be formed by distinctly different pathways (Hinson et al., 1982).

Microsomal P450 enzymes, primarily CYP1A2 and CYP2E1 in mice, are known to play a critical role in the metabolic activation and hepatotoxicity of acetaminophen (Lee et al., 1996; Tonge et al., 1998; Zaher et al., 1998). However, it is not yet clear whether acetaminophen toxicity in extrahepatic tissues depends on hepatic biotransformation. Although hepatic clearance of acetaminophen, mainly through glucuronidation and sulfation pathways (Hjelle and Klaassen, 1984), will probably influence the bioavailability of acetaminophen at extrahepatic tissues, it remains to be determined whether liver-generated acetaminophen metabolites can reach extrahepatic sites through systemic circulation, and then contribute to local toxicity, either directly or after further metabolism in the target tissue. The latter question has been difficult to address because of the lack of in vivo models that permit organ-selective manipulation of the P450 enzymes. We and others (Gu et al., 2003; Henderson et al., 2003) have recently developed one such model (named the liver-specific *Cpr*-null mouse, and referred to as Null mouse in the present article), in which the gene encoding NADPH-cytochrome P450 reductase (*Cpr*), the obligate redox partner for all microsomal P450s, is deleted specifically in the liver. The Null mice were shown to have lost ~95% hepatic microsomal activity in acetaminophen metabolic activation in vitro (Gu et al., 2003) and were resistant to acetaminophen hepatotoxicity in vivo (Henderson et al., 2003). Thus, we should be able to use this novel animal model to study the relative roles of liver and extrahepatic tissues in the metabolic activation and tissue-selective toxicity of acetaminophen and, potentially, any other toxic chemicals that are substrates of microsomal P450.

The aim of this study was to determine whether extrahepatic acetaminophen toxicity is altered in the Null mice. Null and wild-type mice were treated with either identical acetaminophen doses or with differing acetaminophen doses that served to maintain comparable circulating acetaminophen levels in the two strains. The extent of acetaminophen toxicity was determined by measurement of serum alanine aminotransferase (ALT) and by histopathological analysis of known target tissues. The levels of nonprotein sulfhydryl (NPSH) and acetaminophen-protein adduct were also determined in the liver, lung, kidney, and olfactory epithelium (OE). We provide the first evidence that acetaminophen toxicity in the nasal mucosa is not dependent on hepatic microsomal P450-catalyzed metabolic activation, whereas acetaminophen toxicity in the lung, lateral nasal gland (LNG), and kidney depends at least partly on hepatic P450-mediated acetaminophen biotransformation. Our results also demonstrate the feasibility, as well as the associated caveats, of applying the novel Null mouse model for mechanistic studies on tissue-selective toxicity of numerous xenobiotic compounds.

Materials and Methods

Animal Treatment. Protocols for animal breeding and genotyping were reported previously (Wu et al., 2003). Animal-use protocols were approved by the Institutional Animal Care and Use Committee of the Wadsworth Center (Albany, NY). Two- to four-month-old mice from Null and wild-type littermate groups on mixed C57BL/6 (75%) and 129/Sv (25%) genetic background were used in the studies. Animals were normally maintained at 22°C with a 12-h on, 12-h off light cycle and were allowed free access to water and a standard laboratory diet. For acetaminophen clearance experiments, the animals were given a single intraperitoneal injection (at 9:00–10:00 AM, after overnight fasting) of acetaminophen in warm saline (Genter et al., 1998), at a dose of 100, 400, or 600 mg/kg. Animals in the control groups received the vehicle only. For determination of acetaminophen concentrations, the blood samples were collected by tail-bleeding at 15 min and at 1, 2, 6, 8, and 12 h after the injection. For serum ALT level determinations, blood samples were collected 24 h after a single intraperitoneal injection of acetaminophen; for determination of NPSH content and acetaminophen protein adducts formation in various tissues, the animals were killed 2 h after a single intraperitoneal injection of acetaminophen, and the tissues were collected and frozen at −80°C until use.

Histopathological Examination. The mice were killed 24 h after acetaminophen injection. After drawing of blood samples by cardiac puncture, liver, kidney, lung, nose, and heart tissues were collected promptly and fixed in 10% neutral buffered formalin or in Bouin's fixative (for nasal tissues) for histological examination as described previously (Gu et al., 1997, 2003). The nasal cavity was cut at levels 3, 5, and 6 (Young, 1986) to prepare tissue blocks, and sections were made at 2 to 3 μm thickness. The sections were stained with hematoxylin and eosin. For semiquantitative assessment of tissue toxicity, severity of lesions in the tissues was graded with + representing moderate, ± representing mild, and − representing negative.

Determination of Blood Acetaminophen Levels. Blood samples (15 μl each) were collected in heparin-coated capillaries (Drummond Scientific, Broomall, PA) and were mixed with an equal volume of saline. The samples were spun at 800*g* for 5 min at 4°C; the supernatant fractions were mixed with one-half volume of 1 M perchloric acid and spun again at 2000*g* for 5 min to remove precipitated proteins. Aliquots of the final supernatant were analyzed for acetaminophen concentration on a high-performance liquid chromatograph according to the method described previously (Gu et al., 1998).

Determination of Tissue NPSH Contents. Liver, kidney, lung, heart, and OE samples were collected and stored frozen at −80°C before analysis for the content of NPSH, which was determined essentially according to the procedure described by Tonge and coworkers (1998). Glutathione was used as a standard.

Determination of Serum ALT Levels. Three-month-old male mice were fasted overnight before treatment with acetaminophen. Serum ALT levels were determined at 24 h after a single intraperitoneal injection of acetaminophen using a kit from Sigma-Aldrich (DG159-UV; St. Louis, MO), with 10 to 50 μl of serum isolated from blood samples collected by cardiac puncture in individual animals.

Determination of In Vitro Metabolic Activation of Acetaminophen. Metabolic activation of acetaminophen was assayed by a determination of the rates of formation of acetaminophen-glutathione conjugate (GS-AP) (Gu et al., 1998). The reaction mixtures contained 50 mM potassium phosphate, pH 7.6, 0.5 mM acetaminophen, 10 mM reduced glutathione, 1 mM NADPH, and 0.3 to 1.0 mg/ml microsomal or S9 proteins.

Other Methods. Immunoblots were analyzed with an ECL kit from Amersham Biosciences Inc. (Piscataway, NJ), with a goat antibody to rat CYP2E1 (BD Gentest, Bedford, MA) or with a rabbit anti-acetaminophen (4-acetamidobenzoic acid), for detection of acetaminophen protein adducts (Matthews et al., 1996). For acetaminophen protein adduct detection, microsomal and cytosol samples (or S9 for OE) were isolated from the tissues of individual animals, and immunoblot analysis was carried out as described previously (Ding and Coon, 1990) using 10% SDS-polyacrylamide gels, with the anti-acetaminophen serum (1:1000). The putative protein adducts were

identified by comparing the bands detected in the saline-treated mice and the bands detected in the acetaminophen-treated mice; those detected in the saline-treated animals represent nonspecific binding of the antibody to cellular proteins. For CYP2E1 determination, kidney microsomes of 2-month-old male mice were used, and the intensity of the detected band was quantified with a densitometer. Protein concentrations were determined by the bicinchoninic acid method, with bovine serum albumin as the standard (Pierce Chemical, Rockford, IL).

Results

Extrahepatic Tissue Toxicity in the Null and Wild-Type Mice Treated Identically with Acetaminophen. Initial studies of the impact of hepatic metabolism on extrahepatic acetaminophen toxicity were conducted with a known hepatotoxic acetaminophen dose (400 mg/kg i.p.). As expected, hepatotoxicity occurred in wild-type but not in Null mice (Table 1). It is interesting that acetaminophen toxicity was also decreased in three known extrahepatic target tissues—kidney, lung, and nose—of the Null mice compared with the wild-type mice. In the kidney, four of seven wild-type mice demonstrated moderate toxicity, whereas only one of eight Null mice showed mild toxicity. In the lung, all seven wild-type mice showed moderate toxicity, whereas six of eight Null mice exhibited only mild toxicity. Decreases in the severity of toxicity were also seen in various structures in the nose, including the nasal olfactory and respiratory epithelium and the LNG.

Dose-Dependent Differences in Acetaminophen Clearance between the Null and Wild-Type Mice. Pharmacokinetic studies were carried out for a determination of the effects of liver-selective CPR loss on in vivo acetaminophen clearance. As shown in Fig. 1A, at a nontoxic dose of acetaminophen (100 mg/kg i.p.), there was a small but significant decrease ($P < 0.05$) in blood acetaminophen levels in the wild-type mice compared with the Null mice at 1 and 2 h after dosing, although no difference was found in acetaminophen levels at any other time points. In contrast, at a hepatotoxic dose (400 mg/kg), the Null mice demonstrated a faster acetaminophen clearance than did the wild-type mice (Fig. 1B). Acetaminophen blood levels in the Null mice were significantly lower than in the wild-type mice at 6, 8, and 12 h after dosing. At none of these doses was a difference found between the two mouse strains in acetaminophen levels at 15 min after dosing, suggesting that there was no significant difference in acetaminophen absorption between these two strains.

Acetaminophen Toxicity in Liver and Extrahepatic Target Tissues of the Null and Wild-Type Mice Treated with Acetaminophen at Differing Doses Intended to Achieve Equivalent Acetaminophen Blood Levels. The fact that acetaminophen clearance was faster in the Null mice than in the wild-type mice at 400 mg/kg acetaminophen makes it difficult to interpret directly the toxicity data described in Table 1. Further pharmacokinetic studies indicated that after an increase of the acetaminophen dose to 600 mg/kg for the Null mice whereas the acetaminophen dose was maintained at 400 mg/kg for the wild-type mice, an equivalent cumulative blood acetaminophen level was

TABLE 1

Extent of tissue toxicity in the Null and wild-type mice treated with acetaminophen at 400 mg/kg

Male 3-month-old wild-type and Null mice were treated with a single intraperitoneal injection of acetaminophen after overnight fasting. Histopathology of the liver, lung, OE, nasal respiratory epithelium, and LNG was examined 24 h after injection. The severity of lesions in the tissues was graded: +, moderate; ±, mild; and −, negative.

Tissues	Number of Mice in Each Grade					
	Wild-Type			Null		
	+	±	−	+	±	−
Liver	7					8
Kidney	4		3		1	7
Lung	7				6	2
Nose						
OE	5	1	1		3	5
Respiratory epithelium	7			2	6	
LNG	7			2	5	1

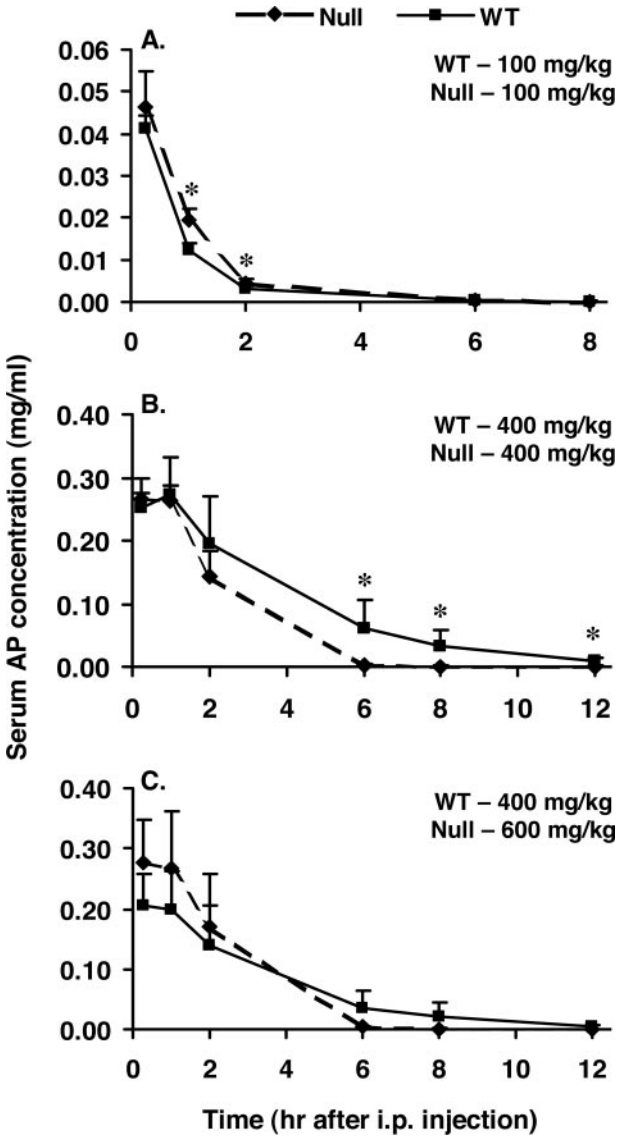

Fig. 1. Acetaminophen clearance in wild-type (WT) and Null mice. Three-month-old male mice were fasted overnight before a single intraperitoneal injection of acetaminophen in saline. Blood samples were collected from individual animals at various times after injection for the determination of serum acetaminophen (AP) concentrations. A, mice of either strain were treated with acetaminophen at 100 mg/kg ($n = 9$ for each strain). B, mice of either strain were treated with acetaminophen at 400 mg/kg ($n = 7$ for WT; $n = 8$ for Null). C, WT and Null mice were treated with acetaminophen at 400 and 600 mg/kg, respectively ($n = 10$ for each strain). Values presented are mean ± S.D. *, $P < 0.05$.

achieved in the two mouse strains (Fig. 1C), with the 0- to 12-h area under the curve being 48.8 ± 20.1 and 48.5 ± 20.4 mg/ml/min for the Null and the wild-type mice, respectively.

The Null mice were still resistant to acetaminophen hepatotoxicity at the 600 mg/kg dose, as indicated by serum ALT levels and histological examination 24 h after acetaminophen injection. In wild-type mice treated with acetaminophen at 400 mg/kg, serum ALT levels reached 9000 ± 4500 U/l (mean ± S.D., $n = 10$), with corresponding hepatic centrilobular necrosis in all individuals examined (Table 2). In contrast, the ALT levels were <200 U/l in the Null mice ($n = 10$) treated with acetaminophen at 600 mg/kg, and no hepatic lesion was present (Table 2).

The Null mice were also protected from acetaminophen toxicity in the kidney (Fig. 2 and Table 2). As shown in Table 2, 7 of 10 wild-type mice had mild to moderate kidney lesions, which were characterized by patchy to diffuse nephrosis with dilated tubules in the cortex (Fig. 2), whereas no lesion was observed in any of the 10 acetaminophen-treated Null mice.

Both Null and wild-type mice showed lung toxicity, which was characterized by the necrosis and detachment of epithelial cells in the small airways, after acetaminophen treatment (Fig. 2 and Table 2). However, the extent of toxicity was greater in the wild-type than in the Null mice (Table 2).

It is interesting that although acetaminophen toxicity in the LNG was also less severe in the Null than in the wild-type mice (Table 2), acetaminophen-induced epithelial necrosis in the OE (Fig. 2) and respiratory epithelium (data not shown) was virtually unaltered by the loss of hepatic CPR (Table 2). Thus, the impact of the loss of hepatic CPR on acetaminophen toxicity in extrahepatic target tissues was seen to be tissue-dependent when the circulating acetaminophen levels were made equivalent for the two strains.

The in vitro acetaminophen-metabolic activity of OE S9 fractions and kidney and lung microsomes was compared between the Null and wild-type male mice (Table 3). No significant difference between the two strains in the rates of formation of GS-AP was found for any of the three tissues. Thus, the local metabolic capacity toward acetaminophen was not decreased in these extrahepatic tissues of the Null mice. In addition, immunoblot analysis indicated that kidney microsomal CYP2E1 protein levels were not significantly different between the Null and the wild-type mice (23 ± 9 and 17 ± 5, respectively, in arbitrary units; $n = 3$, $P > 0.05$).

TABLE 2

Extent of tissue toxicity in the Null mice treated with acetaminophen at 600 mg/kg and in wild-type mice treated with acetaminophen at 400 mg/kg

Male 3-month-old wild-type and Null mice were treated with acetaminophen as described in Table 1 but at a differing dose that was found to produce equivalent serum acetaminophen levels. The severity of lesions in the tissues was graded: +, moderate; ±, mild; and −, negative.

Tissue	Number of Mice in Each Grade					
	Wild-Type (400 mg acetaminophen/kg)			Null (600 mg acetaminophen/kg)		
	+	±	−	+	±	−
Liver	9	1				10
Kidney	2	5	3			10
Lung	9	1		2	8	
Nose						
OE	7	2	1	6	2	2
Respiratory epithelium	9	1		8	2	
LNG	9	1			8	2

Impact of Hepatic CPR Loss on Acetaminophen-Induced Depletion of NPSH in Extrahepatic Target Tissues. NPSH levels were determined (Table 4) as an early indicator of cytotoxicity in the liver and the extrahepatic target tissues of the Null and wild-type mice 2 h after a single intraperitoneal injection of acetaminophen at 400 mg/kg (for wild-type) or 600 mg/kg (for Null). Saline was used as a vehicle control. As expected, acetaminophen treatment caused a ~90% decrease in hepatic NPSH levels in the wild-type mice but only a ~40% decrease in the Null mice, a result consistent with the differential hepatotoxicity seen at 24 h after acetaminophen treatment. It is noteworthy that the NPSH levels in the saline-treated Null mice were significantly lower than those in the saline-treated wild-type mice. The observed decreases in basal NPSH level, which did not occur in the extrahepatic tissues examined, suggest increased oxidative stress in the livers of the Null mice; these livers are known to be fatty and to have focal necrosis (Gu et al., 2003; Henderson et al., 2003).

In the wild-type mice, acetaminophen-induced decreases in NPSH levels were less extensive in the extrahepatic tissues than in the liver (Table 4). In the Null mice, NPSH depletion was not seen in the lung, but it was observed in the kidney and OE. Compared with the extent of NPSH depletion in the wild-type mice, the extents in the kidney and the OE of the Null mice were not significantly different at 2 h after acetaminophen treatment.

Differing Acetaminophen-Protein Adduct Levels in Tissues of the Null and Wild-Type Mice. To determine whether a quantitative or qualitative difference exists between the two mouse strains in the formation of acetaminophen-protein adducts in various tissues after acetaminophen treatment, we used an anti-acetaminophen antibody to detect acetaminophen-protein adducts on immunoblots. Under the immunoblotting conditions used, we detected acetaminophen-protein adducts in liver microsomal and cytosol fractions, and in lung cytosol of the wild-type mice (Fig. 3), but not in kidney or OE microsomal and cytosol fractions (data not shown). Nonspecific bands were detected in all samples, but the presence of these did not interfere with the detection of acetaminophen adducts. The hepatic acetaminophen-protein adducts included a major band (~40 kDa) and several minor bands in the microsomes and two bands in the cytosol. However, these protein adducts were barely detectable in the livers of the Null mice, consistent with the diminished P450 activity in the livers of the Null mice. In lung, only one acetaminophen-protein adduct band (~50 kDa) was detected in the cytosol of the wild-type mice; this band was nearly absent in tissues from the Null mice. Similar patterns of acetaminophen protein adducts were observed in the tissues of female mice and in those of male mice (data not shown).

Discussion

This is the first application of the Null mouse model to studying the role of liver microsomal P450s in extrahepatic chemical toxicity. The tissue-selective loss of hepatic microsomal P450 activities in the Null mouse model has been demonstrated previously (Gu et al., 2003; Henderson et al., 2003). In the present study, we demonstrate for the first time in an animal model that the extrahepatic toxicity of acetaminophen is affected by the activities of liver microsomal

P450s in a tissue-dependent fashion. In the nasal mucosa, which has relatively high levels of microsomal P450 enzymes, and is highly active in the metabolic activation of acetaminophen (Gu et al., 1998), acetaminophen toxicity was not dependent on hepatic microsomal P450-catalyzed metabolic activation. On the other hand, acetaminophen toxicity in the LNG, lung, and kidney, organs with relatively low metabolic activity toward acetaminophen, was reduced or abolished in the Null mice, indicating that the target-tissue toxicity was at least partly caused by acetaminophen metabolites generated in the liver. It is likely that the dependence of extrahepatic toxicity on hepatic biotransformation is different for chemicals activated by differing P450s, because each P450 is believed to have a unique tissue distribution profile. It is noteworthy that acetaminophen, as a model compound for systemically administered toxicants, was given intraperitoneally in this study. However, for the lung and LNG, it will be interesting to determine whether or to what extent liver P450-mediated biotransformation contributes to respiratory-tract toxicities of inhaled chemical compounds, which may have greater bioavailability at the portals of entry.

It should be noted that although the animals used in this study had a mixed genetic background, the two parental mouse strains, C57BL/6 and 129/Sv, are similar to one another in the rates of systemic clearance and in vitro microsomal metabolism of acetaminophen (Zhuo et al., 2004). Therefore, the differences between the Null and wild-type mice in the rates of acetaminophen clearance and the extent of tissue toxicity are unlikely to be confounded by the mixed genetic background of the mice used. In addition, sufficiently large numbers of mice were used in each experimental group that any impact of potential interindividual differences in genetic background would be minimized.

The Null mouse model will be valuable for assessing the role of hepatic biotransformation in the chemical toxicity or efficacy of numerous drugs and other xenobiotic chemicals. However, caution should be exercised in the application of this mouse model before the impact of potential confounding factors has been examined. As illustrated in the present study with acetaminophen, a loss of hepatic P450 activities may directly or indirectly influence systemic clearance of a given compound. For acetaminophen, a slower clearance was found in the Null mice than in the wild-type mice at doses that did not cause hepatotoxicity. However, when a toxic dose was given, acetaminophen was cleared faster in the Null mice than in the wild-type mice, a finding that may be explained by a compensatory induction of phase II enzymes responsible for the conjugation of acetaminophen in the Null mice (Weng et al., 2004), as well as by a probable decrease in acetaminophen metabolism in the livers of the wild-type mice after acetaminophen-induced hepatotoxicity.

The protection from acetaminophen toxicity in the lung,

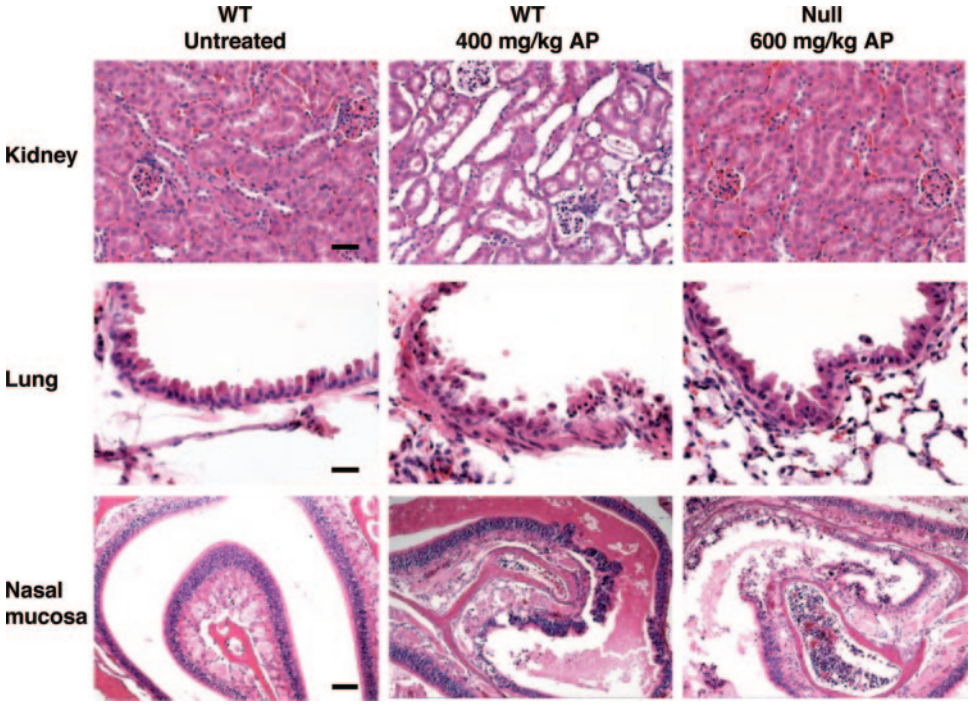

Fig. 2. Tissue-selective impact of hepatic *Cpr* knockout on acetaminophen (AP) toxicity in kidney, lung, and nasal mucosa. Three-month-old male mice were either untreated or treated with a single intraperitoneal injection of acetaminophen in saline at 400 and 600 mg/kg for wild-type (WT) and Null mice, respectively, after overnight fasting. Tissues were obtained for histopathological examination at 24 h after acetaminophen treatment. Representative hematoxylin and eosin-stained sections are shown for kidney, lung, and OE. For the kidney, untreated WT mice had a normal renal cortex with proximal tubules lined by a thick columnar epithelium and distal tubules lined by a lower, cuboidal epithelium; WT mice treated with 400 mg/kg acetaminophen often had mild to moderate, patchy to diffuse nephrosis, and tubules of the cortex were dilated and lined by a low cuboid epithelium, with a few tubules containing eosinophilic material; and Null mice treated with 600 mg/kg acetaminophen displayed a normal renal cortex. For the lung, untreated WT mice exhibited a normal bronchiolar epithelium; WT mice treated with 400 mg/kg acetaminophen showed necrosis and detachment of many epithelial cells of small airway; and Null mice treated with 600 mg/kg acetaminophen showed scattered vacuolated cuboidal epithelial cells with focally pseudostratified epithelium or individual cell necrosis and detachment. For the OE, untreated WT mice had normal ethmoidal turbinates lined by a thick, pseudostratified olfactory neuroepithelium, with Bowman's glands in the submucosa; WT mice treated with 400 mg/kg acetaminophen displayed signs of acute olfactory epithelial injury, including detachment of the mucosa, and the appearance of sloughed cells in the nasal cavity; and Null mice treated with 600 mg/kg acetaminophen also had extensive injury to the OE, evident as epithelial necrosis, detachment, sloughing, and ulceration. Scale bars, 20 μm for kidney, 10 μm for lung, and 40 μm for nasal mucosa.

kidney, and LNG of the Null mice may be explained by a strain-specific absence of liver-generated acetaminophen metabolites, such as GS-AP, in these tissues. Few studies have examined the in vivo distribution of various acetaminophen metabolites, especially for reactive metabolites such as *N*-acetyl-*p*-benzoquinone imine. Nevertheless, it has been suggested that GS-AP contributes to kidney toxicity (Emeigh Hart et al., 1996). Although the strong protection of kidney against acetaminophen toxicity in the Null mice suggests that local metabolic activation is not critical for the renal toxicity, it is possible that the stable liver-generated acetaminophen metabolites, such as GS-AP, are converted back to acetaminophen by local enzymes such as cysteine peptidyl β-lyase (Dekant et al., 1993) and that the regenerated acetaminophen still requires metabolic activation by a local P450 (such as CYP2E1) or non-P450 enzymes to exert renal toxicity. This hypothesis is consistent with data from previous studies that implicate renal CYP2E1 in acetaminophen toxicity (Emeigh Hart et al., 1994; Hoivik et al., 1995), as well as with earlier findings in studies with rats (Newton et al., 1985), which indicated that renal metabolic activation, through the formation of 4-aminophenol, may be important for the nephrotoxicity of acetaminophen. It is noteworthy that the same hepatic metabolites may also reach the nasal mucosa, in which they also contribute to toxicity. However, their amounts may be very small compared with the level of reactive acetaminophen metabolites generated by the nasal mucosal P450s, which make the major contribution to toxicity.

The postactivation mechanisms of acetaminophen toxicity in the liver have been the subject of intense study (Jollow et al., 1973; Rosen et al., 1983; Albano et al., 1985; Cohen et al., 1997). It seems that acetaminophen hepatotoxicity involves multiple mechanisms, including the inactivation of critical cellular protein targets, increased cellular oxidative stress, and mitochondrial dysfunction (Zhou et al., 1996; Cohen et al., 1997; Bruno et al., 1998; Chiu et al., 2002; Knight and Jaeschke, 2002). The mechanisms of acetaminophen toxicity in extrahepatic tissues are less clear than those in the liver. In the kidney, several enzymes, including P450, prostaglandin endoperoxide synthase, and *N*-deacetylase, are believed to be involved in acetaminophen toxicity in the several species that have been examined (Larsson et al., 1985; Newton et al., 1985; Emeigh Hart et al., 1994). An immunohistochemical analysis demonstrated that covalent binding of acetaminophen and the associated tissue damage colocalize with CYP2E1 expression sites in the liver and various extrahepatic tissues (including lung, kidney, and OE) in CD-1 mice (Emeigh Hart et al., 1995), suggesting that toxicity in these tissues was associated with metabolic activation of acetaminophen by local P450s. It has also been postulated that 4-aminophenol, formed through an NADPH-independent deacetylation of acetaminophen, is important for the neph-

TABLE 3

Capacity for acetaminophen metabolic activation was not changed in OE, lung, and kidney of the Null mice

Kidney and lung microsomes and OE S9 fractions of 3- to 4-month-old male mice were analyzed for the rates of formation of GS-AP from acetaminophen (0.5 mM), as described under *Materials and Methods*. Reactions were carried out for 30 min at 37° C. The values represent means ± S.D. ($n = 3$). There was no significant difference between the wild-type and the Null groups for any of the tissues ($P > 0.05$).

Tissue	Rate of Product Formation	
	Wild-Type	Null
	nmol/min/mg protein	
Kidney	0.011 ± 0.002	0.010 ± 0.002
Lung	0.13 ± 0.04	0.10 ± 0.01
OE	0.79 ± 0.19	0.59 ± 0.12

TABLE 4

Effects of acetaminophen treatment on the levels of total nonprotein sulfhydryl in various mouse tissues

Three-month-old male mice were fasted overnight before a single intraperitoneal injection of saline or acetaminophen (AP) at 400 mg/kg for wild-type or 600 mg/kg for Null. Tissues were collected from individual animals at 2 h after injection. The values represent means ± S.D.

Tissue & Treatment	Wild-Type ($n = 11$)		Null ($n = 10$)	
	Total NPSH	AP/Saline	Total NPSH	AP/Saline
	μmol/g of tissue	*%*	*μmol/g of tissue*	*%*
Liver				
Saline	6.5 ± 1.2[a]	11	4.4 ± 0.7	57
AP	0.7 ± 0.3[a,b]		2.5 ± 0.4[b]	
Kidney				
Saline	3.3 ± 0.5	58	3.7 ± 0.6	62
AP	1.9 ± 0.4[b]		2.3 ± 0.5[b]	
Lung				
Saline	1.4 ± 0.3	79	1.2 ± 0.2	100
AP	1.1 ± 0.2[b]		1.2 ± 0.2	
OE				
Saline	1.8 ± 0.3	50	1.9 ± 0.3	42
AP	0.9 ± 0.2[b]		0.8 ± 0.1[b]	

[a] $P < 0.01$, Null versus wild-type mice for a given tissue in each treatment group.
[b] $P < 0.01$, AP versus saline for a given tissue of the same mouse strain.

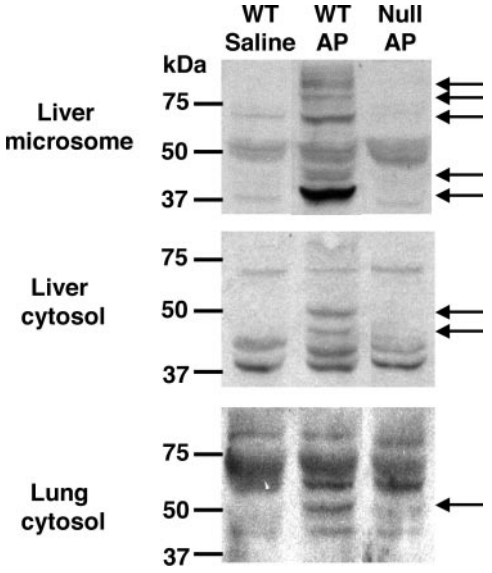

Fig. 3. Immunoblot detection of acetaminophen-protein adducts in liver and lung of acetaminophen-treated mice. Three-month-old female mice were fasted overnight before an intraperitoneal injection of acetaminophen (AP) at 400 mg/kg for wild-type (WT) or 600 mg/kg for Null mice whereas control WT mice were treated with saline. Microsomal and cytosol preparations were obtained from the tissues of individual mice that were killed 2 h after the injection. Liver and lung samples (15 and 10 μg protein, respectively, in each lane) were analyzed on 10% SDS-polyacrylamide gels, and acetaminophen adducts were detected on immunoblots using a polyclonal anti-acetaminophen serum (1:1000). Typical results are shown for three to four mice in each group. The positions of prestained protein molecular weight markers are indicated. Arrows indicate positions of putative acetaminophen adducts. The putative protein adducts were identified by comparing the bands detected in the saline-treated mice and the bands detected in the acetaminophen-treated mice; those detected in the saline-treated animals represent nonspecific binding of the antibody to cellular proteins.

rotoxicity of acetaminophen (Newton et al., 1985); this metabolite, which may initiate redox cycling in the target tissue, was more potent than acetaminophen in the induction of nephrotoxicity.

In our study, the extent of acetaminophen-induced NPSH depletion was significantly decreased in liver and lung of the Null mice, consistent with the decreased severity of tissue damage seen in these organs. Furthermore, acetaminophen-protein adducts were detected in liver and lung of the wild-type mice but was barely detectable in the Null mice after acetaminophen injection. On the other hand, in the kidney and nasal mucosa, the extent of acetaminophen-induced NPSH depletion was similar in the Null and wild-type mice. Acetaminophen-protein adducts were not detected in these tissues under the conditions used with the anti-acetaminophen (4-acetamidobenzoic acid) polyclonal antibody (Matthews et al., 1996), although acetaminophen protein adducts were detected in the kidney in a previous study in which a ^{125}I-conjuagted secondary antibody was used for immunoblot analysis (Bulera et al., 1996). The apparently low abundance of acetaminophen-protein adducts in the kidney and nasal mucosa, tissues that were sustaining damage in the acetaminophen-treated wild-type mice, suggests that protein adduction with acetaminophen is not a major route of acetaminophen toxicity in these organs. Note, however, that the anti-acetaminophen antibody used in our study (Matthews et al., 1996) would not recognize 4-aminophenol or its protein adducts. Therefore, our results do not indicate whether protein adduction with 4-aminophenol or its metabolites occurred in these animals. It should also be noted that the extent of NPSH depletion measured in tissue extracts, as was done in the present study, may not reflect the actual extent of NPSH depletion at the sites most vulnerable to toxicity within an organ, such as OE in the nasal mucosa. Therefore, a correlation between the severity of tissue damage and the extent of NPSH depletion in sensitive cells may exist, but it was not detected in this study.

In summary, we have shown that hepatic P450s play an important role in acetaminophen toxicity in kidney, lung, and the LNG but that they do not contribute to the toxicity in the nasal mucosa. This study demonstrates the value, as well as potential confounding factors, of the Null mouse model for mechanistic studies on extrahepatic toxicity induced by numerous xenobiotic compounds. Note that for a more precise understanding of the role of local metabolism in a given extrahepatic organ, it will be desirable to also study chemical toxicity in mouse models with specific deletion of the *Cpr* gene or a specific P450 gene in the extrahepatic organ, such as the lung or the kidney.

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