Special Contribution

Acetaminophen-Induced Hepatotoxicity: Still an Important Issue

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Acetaminophen (APAP), the most commonly sold over-the-counter antipyretic analgesic, is generally considered harmless at therapeutic doses. However, APAP overdose causes severe and sometimes fatal hepatic damage in humans and experimental animals. In the United States and Europe, the incidence of liver injury due to APAP overdose, either with suicidal intent or by accident, is increasing. Recently, even in Japan, APAP has become more commonly used alone, especially in children with a view to prevention of Reye's syndrome. Thus, understanding the hepatotoxicity induced by APAP overdose is very important. To date, the mechanisms underlying APAP toxicity are considered to be associated with i) covalent binding to cellular macromolecules of a reactive intermediate metabolite of APAP produced by cytochrome P450 (CYP), *N*-acetyl-*p*-benzoquinoneimine, and ii) oxidative stress. APAP-induced hepatotoxicity is modified by associated risk factors such as alcohol abuse, fasting and concomitant drugs. Even therapeutic doses of APAP sometimes induce hepatic damage in the presence of these risk factors. *N*-Acetylcysteine (NAC), one of the cysteine prodrugs, is the most widely used for treating APAP-induced liver injury. In addition to NAC, CYP2E1 inhibitors and antioxidants may also serve as mechanism-based antidotes for APAP overdose. Moreover, inducers of heat shock protein (HSP) (in particular HSP25 and HSP70i) without any side effects might be new-type antidotes.

key words: acetaminophen; *N*-acetyl-*p*-benzoquinoneimine; cytochrome P450 2E1; hepatotoxicity; oxidative stress

Acetaminophen (*N*-acetyl-*p*-aminophenol, APAP), also referred to as paracetamol, is widely used as an analgesic and antipyretic drug throughout the world. In the United Kingdom, about 3.2×10^9 tablets of APAP are consumed every year, which is equivalent to an average of 55 tablets/person (Jones, 1998). As an over-the-counter drug, it can be readily obtained without prescription. Although APAP is generally harmless at therapeutic doses, overdose causes hepatotoxicity (Davidson and Eastham, 1966; Mitchell, 1988). In addition, susceptibility to APAP-induced hepatotoxicity is modified by various factors such as alcohol abuse, fasting and concomitant drug use. In Japan, APAP has usually been consumed as combination tablets or granules and rarely used alone except as a suppository. However, since recent reports that the use of salicylic acid in combination cold remedies is associated with

Reye's syndrome in children with influenza or varicella infection (Belay et al., 1999), APAP has become more likely to be used alone in Japan and in other countries. Therefore, it is important that we review APAP toxicity, even though APAP-induced hepatic injury is not a newly emerging problem.

APAP-induced hepatotoxicity has been demonstrated in experimental animals as well as clinical cases. Mice and hamsters have been shown to be very sensitive to the hepatotoxic effects of APAP, developing a fulminant centrilobular necrosis similar to that observed in humans (Mitchell et al., 1973; Davis et al., 1974; Potter et al., 1974). Rats, rabbits and guinea pigs are, however, relatively resistant to APAP insult (Boyd and Bereczky, 1966; Madhu et al, 1992).

This review focuses on the mechanisms underlying APAP-induced hepatotoxicity, risk factors that increase susceptibility to the hepatotoxic effects of APAP, and protection against the toxicity.

Mechanisms of APAP-Induced Hepatotoxicity

Reactive intermediate metabolite of APAP

APAP is biotransformed and eliminated as nontoxic glucuronic acid and sulfate conjugates. Glucuronide is provided by UDP-glucuronic acid, and sulfation is dependent on phosphoadenylsulfate (Clements et al., 1984). Furthermore, the mixed-function oxidase system cytochrome P450 (CYP) participates in metabolizing a small proportion of APAP at therapeutic doses. The metabolism of APAP by CYP leads to the formation of N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive intermediate metabolite (Dahlin et al., 1984), which is normally detoxified by conjugation with reduced glutathione (GSH).

After high doses of APAP, the capacity for its removal by hepatic conjugation with glucuronide and sulfate is exceeded, and more of the reactive metabolite NAPQI is formed. Consequently, more NAPQI is conjugated with GSH, and when hepatic GSH is depleted, more NAPQI will bind covalently

to cellular macromolecules (Jollow et al., 1973; Potter and Hinson, 1986). This is thought to lead to a loss of protein thiol groups (Moore et al., 1985; Kyle et al., 1990) and ultimately to cell death. In humans, CYP2E1, CYP1A2 and CYP3A4 have been thought to contribute to the metabolism of APAP to form NAPQI (Raucy et al., 1989; Patten et al., 1993; Thummel et al., 1993; Nelson, 1995). However, recent pharmacokinetics studies in human volunteers have demonstrated that involvement of CYP1A2 and CYP3A4 in NAPQI formation in vivo is much less than that of CYP2E1, as omeprazole (CYP1A2 inducer) and rifampicin (CYP3A4 inducer) treatment have no effect on the formation of NAPQI from APAP, whereas disulfiram (CYP2E1 inhibitor) treatment decreases NAPQI formation (Sarich et al., 1997; Manyike et al., 2000). Studies using CYP2E1 and CYP1A2 knockout mice have shown that the former, but not the latter, are more resistant to APAP-induced hepatotoxicity than wild-type animals (Lee et al., 1996; Tonge et al., 1998; Zaher et al., 1998), indicating that CYP2E1 is a primary contributor to APAP biotransformation among CYPs. Concerning the other CYP enzymes responsible for bioactivation of APAP, Hazai et al. (2002) recently reported that selective inhibition of CYP2A6 as well as CYP2E1 significantly decreased NAPQI formation in human liver microsomes, whereas CYP1A2 and CYP3A4 inhibition did not affect NAPQI production, suggesting that CYP2A6 may also be responsible for APAP bioactivation. However, the role of CYP2A6 in APAP metabolism in vivo remains to be established. Overall, it is feasible that the principal CYP responsible for APAP toxicity is CYP2E1.

Oxidative stress

Oxidative stress is also considered to be involved in the induction of hepatotoxicity by APAP. The one-electron oxidation of APAP by CYPs may generate reactive oxygen species (ROS). Hydrogen peroxide and superoxide are produced during metabolic activation of APAP in the mixed function oxidase system (Nordblom and Coon, 1977; Kuthan et al., 1978; de Vries, 1981). It has been reported that

APAP overdose causes decreases in antioxidant enzyme activities such as catalase and glutathione peroxidase (Lores Arnaiz et al., 1995; Chen and Lin, 1997), levels of endogenous antioxidants such as α-tocopherol (α-Toc) and reduced forms of coenzyme Q_9 (CoQ_9H_2) and coenzyme Q_{10} ($CoQ_{10}H_2$) (Amimoto et al., 1995; Sumioka et al., 1998), and the GSH/oxidized glutathione (GSSG) ratio (Jaeschke, 1990; Lores Arnaiz et al., 1995; Amimoto et al., 1995) in animal livers. These endogenous antioxidants, especially α-Toc, CoQ_9H_2 and $CoQ_{10}H_2$ scavenge lipid peroxyl radicals and consequently their own levels decrease (Matsura et al., 1992a, 1992b). Although there are arguments that lipid peroxidation may be a consequence rather than the cause of APAP-induced cell damage (Mitchell et al., 1981, 1984), our time course data obtained by quantitative analysis of biomarkers in mice revealed that the reduction in hepatic CoQ_9H_2 and $CoQ_{10}H_2$ levels preceded increases in the thiobarbituric acid-reactive substance (TBARS) content as an index of lipid peroxidation and plasma alanine aminotransferase (ALT) activity following APAP treatment (Amimoto et al, 1995). Moreover, pretreatment with oxidized coenzyme Q_{10} (CoQ_{10}) resulted in an increase in hepatic $CoQ_{10}H_2$ and a marked reduction in hepatic TBARS content and plasma ALT activity without affecting hepatic GSH after APAP injection (Amimoto et al., 1995). Pretreatment with α-Toc also suppressed the increase in the hepatic TBARS content and plasma ALT activity without affecting the hepatic GSH level (Amimoto et al., 1995). These results strongly suggest that lipid peroxidation is involved in the mechanism of APAP-induced hepatic injury.

Recently, it has been reported that the formation of reactive nitrogen species such as peroxynitrite followed by extensive protein nitration is also associated with APAP-induced hepatotoxicity (Hinson et al., 1998; Knight et al., 2001).

Risk Factors

Even therapeutic doses of APAP sometimes induce hepatotoxicity in the presence of risk factors (such as alcohol abuse, fasting and drug interaction), which increase susceptibility to the hepatotoxic effects of APAP.

Alcohol

Interaction between alcohol and APAP has been recognized since the late 1970s (McClain et al., 1980). However, the APAP-alcohol interaction is very complicated, because acute and chronic alcohol intakes have opposite effects. Chronic alcohol intake increases hepatic CYP2E1 activity (Perrot et al., 1989) and decreases the GSH level (Lauterburg and Velez, 1988), especially the liver mitochondrial GSH level (Hirano et al., 1992; Zhao et al., 2002; Zhao and Slattery 2002). These changes lead to an increase in NAPQI formation and a decrease in NAPQI detoxification, resulting in accumulation of NAPQI. Consequently, the severity of APAP-induced hepatotoxicity is enhanced by chronic alcohol intake (Sato et al., 1981a; Zimmerman and Maddrey, 1995; Schmidt et al., 2002), and even a therapeutic dose of APAP (generally considered to be nontoxic in nonalcoholics) may lead to hepatotoxicity. On the other hand, acute alcohol intake reduces the severity of APAP-induced hepatotoxicity (Rumack et al., 1981; Banda and Quart 1982). In rats, this effect may be due to direct inhibition by alcohol of the biotransformation of APAP to NAPQI (Sato et al., 1981b, 1981c). Because alcohol itself is metabolized not only by alcohol dehydrogenase but also by CYP2E1 (microsomal ethanol oxidizing system; MEOS) and MEOS plays a major role when blood ethanol levels are high (Lieber, 1990), alcohol may competitively inhibit biotransformation of APAP to NAPQI. In these studies, APAP was administered when ethanol was still present in the blood circulation. Because ethanol is both a substrate and inhibitor of CYP2E1 (Lieber, 1997), the relative timing of APAP and ethanol ingestion is critical when attempting to elucidate the effect of short-term ethanol exposure on APAP metabolism. Recently, Thummel et al. (2000) reported, from studies in healthy adult volunteers, that the increase in NAPQI formation caused by ethanol occurred only when APAP was given after ethanol had cleared from the body.

Table 1. Protective effect of cysteine prodrugs on APAP-induced hepatotoxicity

Compound	Experimental model	References	
N-Acetylcysteine	Mouse hepatocytes (in vitro)	Massey and Racz	1981
	Rats (in vivo)	Lauterburg et al.	1983
	Mice (in vivo)	Corcoran et al.	1985
	Mice (in vivo)	Hjelle et al.	1986
	Mice (in vivo)	Corcoran and Wong	1986
L-2-Oxothiazolidine-4-carboxylate L-2-Methylthiazolidine-4-carboxylate	Mice (in vivo)	Hazelton et al.	1986
2-(Polyhydroxyalkyl)thiazolidine-4(R)-carboxylic acids	Mice (in vivo)	Roberts et al.	1987
Cystathionine	Mice (in vivo)	Kitamura et al.	1989
2-Methyl-thiazolidine-2,4-dicarboxylic acid	HepG2 cells (in vitro)	Wlodek and Rommelspacher	1997a
	Mice (in vivo)	Wlodek and Rommelspacher	1997b
Ribose cysteine	Mice (in vivo)	Roberts et al.	1992
	Mice (in vivo)	Lucas et al.	2000
2(R,S)-n-Propylthiazolidine-4(R)-carboxylic acid	Mice (in vivo)	Srinivasan et al.	2001
N,S-bis-Acetyl-L-cysteine	Mice (in vivo)	Crankshaw et al.	2002

Fasting

Fasting has also been considered a risk factor for APAP-induced hepatotoxicity. Whitcomb and Block (1994) reported a clinical study which found that APAP-induced hepatotoxicity after an overdose is more likely to be associated with recent fasting than recent alcohol use. In rats, fasting causes severe malnutrition, and the capacity of hepatic conjugation of APAP with glucuronide and sulfate decreases (Price et al., 1986, 1987). Consequently, more APAP is metabolized by CYPs, resulting in more NAPQI production. Although NAPQI is detoxified by conjugation with GSH, fasting also decreases hepatic GSH (Langley and Kelly, 1992; Vogt and Richie, 1993). Therefore, fasting may potentiate APAP-induced hepatotoxicity.

Drug interactions

Finally, long-term treatment with drugs that induce CYPs may increase the risk of APAP-induced liver damage. Carbamazepine, phenytoine, isoniazid and troglitazone are considered to induce CYPs, and therefore long-term use of these drugs enhances APAP-induced hepatotoxicity (Smith et al., 1986;

Minton et al., 1988; Crippin, 1993; Li et al., 2002). In contrast, susceptibility to APAP liver injury is decreased by pretreatment with CYP inhibitors such as piperonyl butoxide and 4-methylpyrazone (Brady et al., 1991; Brennan et al., 1994; Kucukardali et al., 2002, Hazai et al., 2002).

Protection against APAP-Induced Hepatotoxicity

Cysteine prodrugs

GSH plays an important role in protecting the liver against APAP-induced hepatotoxicity, because NAPQI is detoxified by conjugation with GSH. Therefore, stimulation of hepatic GSH synthesis is feasible for prevention of APAP-induced hepatic injury. GSH, a tripeptide comprising glutamate, cysteine and glycine, is synthesized by a two-step reaction. The first step is catalyzed by γ-glutamyl-cysteine synthase to form γ-glutamylcysteine. In the second step, GSH synthase catalyzes the reaction between glycine and γ-glutamylcysteine to form GSH (Wang and Ballatori, 1998). The first step catalyzed by γ-glutamylcysteine synthase

(rate-limiting enzyme) is regulated by feedback inhibition of GSH (Richman and Meister, 1975). When GSH is consumed and the regulation of feedback inhibition is lost, the availability of cysteine as a precursor can become the limiting factor for GSH synthesis (Wang and Ballatori, 1998).

Various cysteine prodrugs have been reported to protect the liver from APAP-induced hepatotoxicity in experiments using animals or cultured cells (Table 1). The mechanism responsible for this protection may be metabolism of these prodrugs to L-cysteine, which is incorporated into hepatic GSH (Lauterburg et al., 1983; Corcoran and Wong, 1986; Hazelton et al., 1986; Roberts et al., 1987).

Among these cysteine prodrugs, *N*-acetylcysteine (NAC) is the most widely used antidote for APAP overdose in clinical practice. Intravenous administration of NAC to patients with APAP poisoning is effective for preventing APAP-induced hepatotoxicity (Prescott, 1981; Smilkstein et al., 1991). Prescott (1981) reported that NAC given intravenously 8 to 10 h after APAP ingestion was effective for preventing liver damage, hepatic failure, renal damage and death. Oral administration of NAC to patients with APAP poisoning was also shown to be effective for preventing liver damage (Rumack et al., 1981; Smilkstein et al., 1988). Analysis of the national multicenter study (1976 to 1985) in the United States reported by Smilkstein et al. (1988) demonstrated that oral treatment with NAC was protective regardless of the initial plasma APAP concentration when given within 8 h of APAP ingestion. They concluded that NAC treatment should be started within 8 h of APAP ingestion, but that treatment is still indicated at least as late as 24 h after ingestion. As shown in Fig. 1, we demonstrated that oral treatment of mice with NAC 1 h after APAP administration significantly suppressed the increase in plasma ALT activity as an index of liver injury. The most appropriate route and dose regimen of NAC for APAP-induced hepatotoxicity is still controversial. However, retrospective and prospective studies suggest that NAC treatment may be effective for fulminant liver failure after APAP overdose (Harrison et al., 1990; Keays et al., 1991).
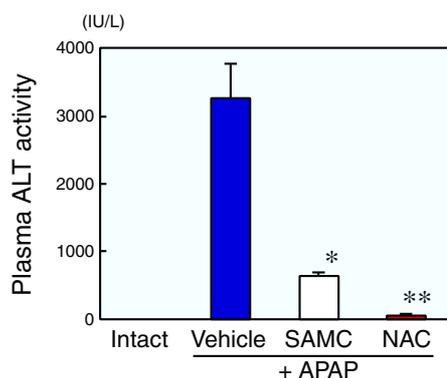
Fig. 1. Effect of SAMC or NAC treatment on APAP-induced liver injury in male ddY mice. APAP (500 mg/kg) was orally administered to mice, and then SAMC (100 mg/kg) or NAC (80 mg/kg) was given orally 1 h after APAP administration. The mice were anesthetized with diethyl ether and blood samples were taken from the right ventricle with a heparinized syringe 6 h after APAP. Plasma ALT activity was determined spectrophotometrically with a commercially available kit. The data are expressed as means ± SE for 4 to 5 animals. Plasma ALT activity in intact mice was 8 ± 1 IU/L (Note: these values are too small to visualize). *,** Significantly different from the APAP + vehicle group ($P < 0.05$ and 0.01, respectively). Unpublished data, Sumioka et al.

CYP2E1 inhibitors

The metabolism of APAP by CYP leads to the formation of NAPQI as discussed in the "Reactive intermediate metabolite of APAP" section. Therefore, CYP inhibitors may be effective for APAP-induced hepatotoxicity. Of the CYP enzymes, CYP2E1 plays the most important role in metabolizing APAP. Studies of CYP2E1 knockout mice have revealed that CYP2E1 is the most important factor in APAP biotransformation (Lee et al., 1996; Zaher et al., 1998).

We have demonstrated that *S*-allylmercaptocysteine (SAMC), an organosulfur compound in aged garlic extract, protects mice from APAP-induced liver injury. Oral treatment with SAMC (100–200 mg/kg) suppressed the increase in plasma ALT activity (Fig. 1) and the hepatic necrosis after APAP overdose, and reduced APAP-induced mortality. The mechanism underlying this protection involved the suppression of CYP2E1 activity

(Nakagawa et al., 1989; Sumioka et al., 1998, 2001). Diallyl sulfide and phenethyl isothiocyanate, compounds derived from garlic and cruciferous plants, respectively, have also been shown to inhibit CYP2E1 activity, and thereby to exhibit a protective effect against APAP-induced hepatotoxicity (Hu et al., 1996; Li et al., 1997). A synthetic compound, 2-(allylthio)pyradine, inhibited CYP2E1 mRNA and protein expression, resulting in prevention of APAP-induced liver damage (Kim et al., 1997). SAMC or diallyl sulfide treatment, not only before but also after APAP overdose, can protect animals from APAP-induced hepatotoxicity (Hu et al., 1996; Sumioka et al., 2001), suggesting that CYP2E1 inhibitor could serve as an antidote for APAP overdose.

CYP2E1 is also known to be a key bioactivator of various carcinogens, such as azoxymethane and N-nitrosodimethylamine (Yoo et al., 1990; Sohn et al., 2001). In this context, a CYP2E1 inhibitor could be a potential inhibitor of carcinogenesis by environmental carcinogens (Smith et al., 1995), and its clinical application could be extended from
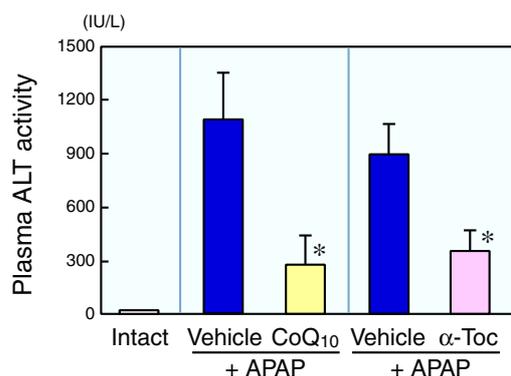
Fig. 2. Effect of CoQ_{10} or α-Toc pretreatment on APAP-induced liver injury in male ICR mice. CoQ_{10} (5 mg/kg), α-Toc (20 mg/kg) or vehicle was intravenously injected into mice 12 h before intraperitoneal administration of APAP (400 mg/kg). The mice were anesthetized with diethyl ether and blood samples were taken from the right ventricle with a heparinized syringe 3 h after APAP. Plasma ALT activity was determined spectrophotometrically with a commercially available kit. The data are expressed as means ± SE for at least 5 animals. * Significantly different from the APAP + vehicle group ($P < 0.05$). From Amimoto et al., 1995 with some modifications.

treatment of APAP-induced hepatotoxicity to treatment of all CYP2E1-related disorders.

Antioxidants

Oxidative stress followed by lipid peroxidation is also thought to contribute to the initiation or progression of APAP-induced hepatotoxicity (Wendel et al., 1979; Albano et al., 1983, 1985), as described in the "Oxidative stress" section.

We have demonstrated that intravenous pretreatment with the lipid-soluble antioxidants, CoQ_{10} (5 mg/kg) and α-Toc (20 mg/kg), can limit hepatic injury produced by APAP overdose (Amimoto et al., 1995). CoQ_{10} is converted to $CoQ_{10}H_2$ in liver cells, and thereafter acts as an antioxidant (Matsura et al., 1992a, 1992b). These lipid-soluble antioxidants suppressed the increase in plasma ALT activity (Fig. 2) and hepatic TBARS level without affecting the hepatic GSH level. L-Ascorbic acid and its esters (Raghuram et al., 1978; Lake et al., 1981; Mitra et al., 1991) and β-carotene (Baranowitz and Maderson, 1995) also exert protective action against APAP-induced hepatotoxicity. In addition, antioxidant enzymes such as superoxide dismutase and catalase protect the liver from APAP-induced injury (Kyle et al., 1987; Nakae et al., 1990). Iron is well known to play a major role in lipid peroxidation (Halliwell and Gutteridge, 1990). Sakaida et al. (1995) reported that deferoxamine, an iron chelator, can protect against APAP-induced hepatotoxicity.

Given that these antioxidants are effective when administered before APAP, taking APAP with them might be helpful for protecting against toxicity by misadventure, especially as antioxidants in nature, such as CoQ_{10}, α-Toc, ascorbic acid and β-carotene, have few side effects.

Heat shock protein (HSP) inducers

HSP is ubiquitous in nature, and expressed in both prokaryotic and eukaryotic cells in response to a variety of stresses (Hendrick and Hartl, 1993). Initially, HSP was identified as a cellular response protein to hyperthermia, but HSP induction has also been observed after treatment of cells with a num-
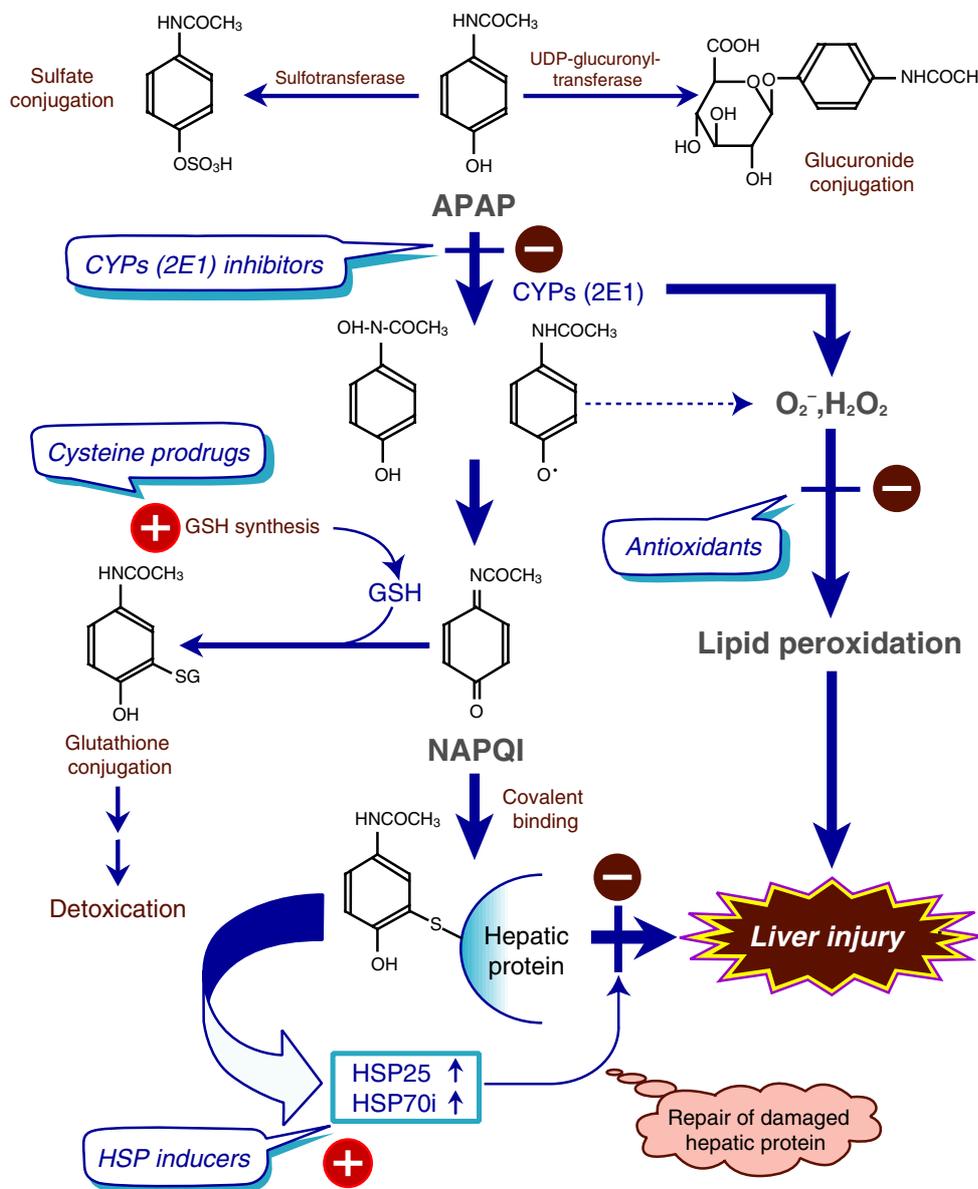
Fig. 3. The metabolic pathways of APAP, and the mechanisms of, and protection against, APAP-induced hepatotoxicity. +, stimulation; –, inhibition.

ber of chemical toxicants. APAP-induced hepatotoxicity has been shown to increase the expression of HSP25 and HSP70i (Salminen et al., 1997a). It is generally accepted that the stimulus for increased HSP synthesis in response to stresses is the presence of non-native proteins (Ananthan et al., 1986; Goff and Goldberg, 1985). In the case of APAP-induced hepatotoxicity, covalent binding of NAPQI to hepatic macromolecules is thought to play a pivotal role in triggering HSP induction (Salminen et al., 1998). HSP70i together with HSP25 may function as a cytoprotective HSP to repair damaged protein in the necrotic lesion. The patterns of hepatic HSP25 and HSP70i induction are clearly different. The level of HSP70i increases initially after APAP overdose, and thereafter the level of HSP25 increases. The induction time after APAP overdose may differ with the severity of hepatotoxicity. For

example, at a lethal dose, HSP25 could be detected 24 h after APAP treatment (Sumioka et al., 2004), whereas, at a necrotic but not lethal dose, it was detectable 6 h after APAP treatment (Salminen et al., 1997a). Salminen et al. (1997b) reported that intraperitoneal pretreatment with amphetamine (15 mg/kg) in mice causes an acute rise in core body temperature to 40°C for at least 1 h, increases the levels of HSP25 and HSP70i, and protects the liver from APAP-induced hepatotoxicity. We have reported that the level of expression of hepatic HSP25 may be a crucial determinant of the fate of mice exposed to APAP insult, because mortality following APAP overdose declined rapidly after the appearance of hepatic HSP25 (Sumioka et al., 2004). These findings suggest that HSP (HSP25 and 70i) inducer might be a new type of antidote for APAP overdose.

Conclusion

APAP overdose causes hepatotoxicity, which is sometimes fatal. This hepatotoxicity is initiated by the metabolic activation of APAP to NAPQI, a reactive metabolite. ROS generation and subsequent lipid peroxidation during APAP biotransformation is likely to exacerbate APAP insult. Even at therapeutic doses, APAP hepatotoxicity can occur when there are risk factors such as alcohol abuse, fasting and concomitant drug use. NAC, one of the cysteine prodrugs, has been traditionally used to prevent the development of hepatotoxicity following a significant overdose of APAP. In addition to NAC, HSP inducers as well as CYP2E1 inhibitors and antioxidants may also be antidotes for APAP overdose, on the basis of the mechanisms underlying APAP toxicity (Fig. 3).

References

1 Albano E, Poli G, Chiarpotto E, Biasi F, Dianzani MU. Paracetamol-stimulated lipid peroxidation in isolated rat and mouse hepatocytes. Chem Biol Interact 1983; 47:249–263.
2 Albano E, Rundgren M, Harvison PJ, Nelson SD, Moldeus P. Mechanisms of N-acetyl-p-benzoquinone imine cytotoxicity. Mol Pharmacol 1985;28:306–311.
3 Amimoto T, Matsura T, Koyama S, Nakanishi T, Yamada K, Kajiyama G. Acetaminophen-induced hepatic injury in mice: the role of lipid peroxidation and effects of pretreatment with coenzyme Q_{10} and α-tocopherol. Free Radic Biol Med 1995;19:169–176.
4 Ananthan J, Goldberg AL, Voellmy R. Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. Science 1986;232: 522–524.
5 Banda PW, Quart BD. The effect of mild alcohol consumption on the metabolism of acetaminophen in man. Res Commun Chem Pathol Pharmacol 1982;38:57–70.
6 Baranowitz SA, Maderson PF. Acetaminophen toxicity is substantially reduced by beta-carotene in mice. Int J Vitam Nutr Res 1995;65:175–180.
7 Belay ED, Bresee JS, Holman RC, Khan AS, Shahriari A, Schonberger LB. Reye's syndrome in the United States from 1981 through 1997. N Engl J Med 1999; 340:1377–1382.
8 Boyd EM, Bereczky GM. Liver necrosis from paracetamol. Br J Pharmacol 1966;26:606–614.
9 Brady JT, Birge RB, Khairallah EA, Cohen SD. Post-treatment protection with piperonyl butoxide against acetaminophen hepatotoxicity is associated with changes in selective but not total covalent binding. Adv Exp Med Biol 1991;283:689–692.
10 Brennan RJ, Mankes RF, Lefevre R, Raccio-Robak N, Baevsky RH, DelVecchio JA, et al. 4-Methylpyrazole blocks acetaminophen hepatotoxicity in the rat. Ann Emerg Med 1994;23:487–494.
11 Chen LH, Lin SM. Modulation of acetaminophen-induced alterations of antioxidant defense enzymes by antioxidants or glutathione precursors in cultured hepatocytes. Biochem Arch 1997;13:113–125.
12 Clements JA, Critchley JA, Prescott LF. The role of sulphate conjugation in the metabolism and disposition of oral and intravenous paracetamol in man. Br J Clin Pharmacol 1984;18:481–485.
13 Corcoran GB, Racz WJ, Smith CV, Mitchell JR. Effects of N-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. J Pharmacol Exp Ther 1985;232:864–872.
14 Corcoran GB, Wong BK. Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by N-acetyl-L-cysteine in vivo: studies with N-acetyl-D-cysteine in mice. J Pharmacol Exp Ther 1986;238:54–61.
15 Crankshaw DL, Berkeley LI, Cohen JF, Shirota FN, Nagasawa HT. Double-prodrugs of L-cysteine: differential protection against acetaminophen-induced hepatotoxicity in mice. J Biochem Mol Toxicol 2002;16: 235–244.
16 Crippin JS. Acetaminophen hepatotoxicity: potentiation by isoniazid. Am J Gastroenterol 1993;88:590–592.
17 Dahlin DC, Miwa GT, Lu AYH, Nelson SD. N-Acetyl-p-benzoquinone imine: a cytochrome P-450-mediated

oxidation product of acetaminophen. Proc Natl Acad Sci USA 1984;81:1327–1331.

18 Davidson DG, Eastham WN. Acute liver necrosis following overdose of paracetamol. Br Med J 1966; 5512:497–499.

19 Davis DC, Potter WZ, Jollow DJ, Mitchell JR. Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. Life Sci 1974;14:2099–2109.

20 de Vries J. Hepatotoxic metabolic activation of paracetamol and its derivatives phenacetin and benorilate: oxygenation or electron transfer? Biochem Pharmacol 1981;30:399–402.

21 Goff SA, Goldberg AL. Production of abnormal proteins in *E. coli* stimulates transcription of lon and other heat shock genes. Cell 1985;41:587–595.

22 Halliwell B, Gutteridge MC. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 1990;186:1–85.

23 Harrison PM, Keays R, Bray GP, Alexander GJ, Williams R. Improved outcome of paracetamol-induced fulminant hepatic failure by late administration of acetylcysteine. Lancet 1990;335:1572–1573.

24 Hazai E, Vereczkey L, Monostory K. Reduction of toxic metabolite formation of acetaminophen. Biochem Biophys Res Commun 2002;291:1089–1094.

25 Hazelton GA, Hjelle JJ, Klaassen CD. Effects of cysteine pro-drugs on acetaminophen-induced hepatotoxicity. J Pharmacol Exp Ther 1986;237:341–349.

26 Hendrick JP, Hartl F. Molecular chaperone functions of heat-shock proteins. Annu Rev Biochem 1993;62: 349–384.

27 Hinson JA, Pike SL, Pumd NR, Mayeux PR. Nitrotyrosine protein adducts in hepatic centrilobular areas following toxic doses of acetaminophen in mice. Chem Res Toxicol 1998;11:604-607.

28 Hirano T, Kaplowitz N, Tsukamoto H, Kamimura S, Fernandez-Checa JC. Hepatic mitochondrial glutathione depletion and progression of experimental alcoholic liver disease in rats. Hepatology 1992;16:1423–1427.

29 Hjelle JJ, Brzeznicka EA, Klaassen CD. Comparison of the effects of sodium sulfate and *N*-acetylcysteine on the hepatotoxicity of acetaminophen in mice. J Pharmacol Exp Ther 1986;236:526–534.

30 Hu JJ, Yoo JS, Lin M, Wang EJ, Yang CS. Protective effects of diallyl sulfide on acetaminophen-induced toxicities. Food Chem Toxicol 1996;34:963–969.

31 Jaeschke H. Glutathione disulfide formation and oxidant stress during acetaminophen-induced hepatotoxicity in mice in vivo: the protective effect of allopurinol. J Pharmacol Exp Ther 1990;255:935–941.

32 Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. J Pharmacol Exp Ther 1973;187:195–202.

33 Jones AL. Mechanism of action and value of *N*-acetylcysteine in the treatment of early and late acetaminophen poisoning: a critical review. J Toxicol Clin Toxicol 1998;36:277–285.

34 Keays R, Harrison PM, Wendon JA, Forbes A, Gove C, Alexander GJ, et al. Intravenous acetylcysteine in paracetamol induced fulminant hepatic failure: a prospective controlled trial. BMJ 1991;303:1026–1029.

35 Kim ND, Kwak MK, Kim SG. Inhibition of cytochrome P450 2E1 expression by 2-(allylthio) pyrazine, a potential chemoprotective agent: hepatoprotective effects. Biochem Pharmacol 1997;53:261–269.

36 Kitamura Y, Kamisaki Y, Itoh T. Hepatoprotective effects of cystathionine against acetaminophen-induced necrosis. J Pharmacol Exp Ther 1989;250:667–671.

37 Knight TR, Kurtz A, Bajt ML, Hinson JA, Jaeschke H. Vascular and hepatocellular peroxynitrite formation during acetaminophen-induced liver injury: role of mitochondrial oxidant stress. Toxicol Sci 2001;62: 212–220.

38 Kucukardali Y, Cinan U, Acar HV, Ozkan S, Top C, Nalbant S, et al. Comparison of the therapeutic efficacy of 4-methylpyrazole and *N*-acetylcysteine on acetaminophen (paracetamol) hepatotoxicity in rats. Curr Med Res Opin 2002;18:78–81.

39 Kuthan H, Tsuji H, Graf H, Ullrich V. Generation of superoxide anion as a source of hydrogen peroxide in a reconstituted monooxygenase system. FEBS Lett 1978;91: 343–345.

40 Kyle ME, Miccadei S, Nakae D, Farber JL. Superoxide dismutase and catalase protect cultured hepatocytes from the cytotoxicity of acetaminophen. Biochem Biophys Res Commun 1987;149:889–896.

41 Kyle ME, Sakaida I, Serroni A, Farber JL. Metabolism of acetaminophen by cultured rat hepatocytes. Depletion of protein thiol groups without any loss of viability. Biochem Pharmacol 1990;40:1211–1218.

42 Lake BG, Harris RA, Phillips JC, Gangolli SD. Studies on the effects of L-ascorbic acid on acetaminophen-induced hepatotoxicity. 1. Inhibition of the covalent binding of acetaminophen metabolites to hepatic microsomes in vitro. Toxicol Appl Pharmacol 1981;60:229–240.

43 Langley SC, Kelly FJ. Differing response of the glutathione system to fasting in neonatal and adult guinea pigs. Biochem Pharmacol 1992;44:1489–1494.

44 Lauterburg BH, Corcoran GB, Mitchell JR. Mechanism of action of *N*-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in vivo. J Clin Invest 1983;71:980–991.

45 Lauterburg BH, Velez ME. Glutathione deficiency in alcoholics: risk factor for paracetamol hepatotoxicity. Gut 1988;29:1153–1157.

46 Lee SST, Buters JTM, Pineau T, Fernandez-Salguer P, Gonzalez FJ. Role of CYP2E1 in the hepatotoxicity of acetaminophen. J Biol Chem 1996;271:12063–12067.

47 Li J, Kaneko T, Wang Y, Qin LQ, Wang PY, Sato A. Troglitazone enhances the hepatotoxicity of acetaminophen by inducing CYP3A in rats. Toxicology 2002;176: 91–100.

48 Li Y, Wang EJ, Chen L, Stein AP, Reuhl KR, Yang CS. Effects of phenethyl isothiocyanate on acetaminophen

metabolism and hepatotoxicity in mice. Toxicol Appl Pharmacol 1997;144:306–314.

49 Lieber CS. Mechanism of ethanol induced hepatic injury. Pharmac Ther 1990;46:1–41.

50 Lieber CS. Cytochrome P-450 2E1: its physiological and pathological role. Physiol Rev 1997;77:517–544.

51 Lores Arnaiz S, Llesuy S, Cutrin JC, Boveris A. Oxidative stress by acute acetaminophen administration in mouse liver. Free Radic Biol Med 1995;19:303–310.

52 Lucas AM, Hennig G, Dominick PK, Whiteley HE, Roberts JC, Cohen SD. Ribose cysteine protects against acetaminophen-induced hepatic and renal toxicity. Toxicol Pathol 2000;28:697–704.

53 Madhu C, Maziasz T, Klaassen CD. Effect of pregnenolone-16a-carbonitrile and dexamethasone on acetaminophen-induced hepatotoxicity in mice. Toxicol Appl Pharmacol 1992;115:191–198.

54 Manyike PT, Kharasch ED, Kalhorn TF, Slattery JT. Contribution of CYP2E1 and CYP3A to acetaminophen reactive metabolite formation. Clin Pharmacol Ther 2000;67:275–282.

55 Massey TE, Racz WJ. Effects of N-acetylcysteine on metabolism, covalent binding, and toxicity of acetaminophen in isolated mouse hepatocytes. Toxicol Appl Pharmacol 1981;60:220–228.

56 Matsura T, Yamada K, Kawasaki T. Difference in antioxidant activity between reduced coenzyme Q_9 and reduced coenzyme Q_{10} in the cell: studies with isolated rat and guinea pig hepatocytes treated with a water-soluble radical initiator. Biochim Biophys Acta 1992a; 1123:309–315.

57 Matsura T, Yamada K, Kawasaki T. Antioxidant role of cellular reduced coenzyme Q homologs and α-tocopherol in free radical-induced injury of hepatocytes isolated from rats fed diets with different vitamin E contents. Biochim Biophys Acta 1992b;1127:277–283.

58 McClain CJ, Kromhout JP, Peterson FJ, Holtzman JL. Potentiation of acetaminophen hepatotoxicity by alcohol. JAMA 1980;244:251–253.

59 Minton NA, Henry JA, Frankel RJ. Fatal paracetamol poisoning in an epileptic. Hum Toxicol 1988;7:33–34.

60 Mitchell JR. Acetaminophen toxicity. N Engl J Med 1988;319:1601–1602.

61 Mitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J Pharmacol Exp Ther 1973;187:185–194.

62 Mitchell JR, Smith CV, Hughes H, Lauterburg BH, Horning MG. Overview of alkylation and peroxidation mechanisms in acute lethal hepatocellular injury by chemically reactive metabolites. Semin Liver Dis 1981;1: 143–150.

63 Mitchell JR, Smith CV, Lauterburg BH, Hughes H, Corcoran GB, Horning EC. Reactive metabolites and the pathophysiology of acute lethal cell injury. In: Mitchell JR, Horning MG, eds. Drug metabolism and drug toxicity. New York: Raven; 1984. p. 301–319.

64 Mitra A, Kulkarni AP, Ravikumar VC, Bourcier DR.

Effect of ascorbic acid esters on hepatic glutathione levels in mice treated with a hepatotoxic dose of acetaminophen. J Biochem Toxicol 1991;6:93–100.

65 Moore M, Thor H, Moore G, Nelson S, Moldéus P, Orrenius S. The toxicity of acetaminophen and N-acetyl-p-benzoquinone imine in isolated hepatocytes is associated with thiol depletion and increased cytosolic Ca^{2+}. J Biol Chem 1985;260:13035–13040.

66 Nakae D, Yamamoto K, Yoshiji H, Kinugasa T, Maruyama H, Farber JL, et al. Liposome-encapsulated superoxide dismutase prevents liver necrosis induced by acetaminophen. Am J Pathol 1990;136:787–795.

67 Nakagawa S, Kasuga S, Matsuura H. Prevention of liver damage by aged garlic extract and its components in mice. Phytother Res 1989;3:50–53.

68 Nelson SD. Mechanisms of the formation and disposition of reactive metabolites that can cause acute liver injury. Drug Metab Rev 1995;27:147–177.

69 Nordblom GD, Coon MJ. Hydrogen peroxide formation and stoichiometry of hydroxylation reactions catalyzed by highly purified liver microsomal cytochrome P-450. Arch Biochem Biophys 1977;180:343–347.

70 Patten CJ, Thomas PE, Guy RL, Lee M, Gonzalez FJ, Guengerich FP, et al. Cytochrome P450 enzymes involved in acetaminophen activation by rat and human liver microsomes and their kinetics. Chem Res Toxicol 1993;6:511–518.

71 Perrot N, Nalpas B, Yang CS, Beaune PH. Modulation of cytochrome P450 isozymes in human liver, by ethanol and drug intake. Eur J Clin Invest 1989;19:549–555.

72 Potter WZ, Thorgeirsson SS, Jollow DJ, Mitchell JR. Acetaminophen-induced hepatic necrosis. V. Correlation of hepatic necrosis, covalent binding and glutathione depletion in hamsters. Pharmacology 1974;12: 129–143.

73 Potter DW, Hinson JA. Reactions of N-acetyl-p-benzoquinone imine with reduced glutathione, acetaminophen, and NADPH. Mol Pharmacol 1986;30:33–41.

74 Prescott LF. Treatment of severe acetaminophen poisoning with intravenous acetylcysteine. Arch Intern Med 1981;141:386–389.

75 Price VF, Miller MG, Jollow DJ. Mechanisms of fasting-induced potentiation of acetaminophen hepatotoxicity in the rat. Biochem Pharmacol 1987;36:427–433.

76 Price VF, Schulte JM, Spaethe SM, Jollow DJ. Mechanism of fasting-induced suppression of acetaminophen glucuronidation in the rat. Adv Exp Med Biol 1986; 197:697–706.

77 Raghuram TC, Krishnamurthi D, Kalamegham R. Effect of vitamin C on paracetamol hepatotoxicity. Toxicol Lett 1978;2:175–178.

78 Raucy JL, Lasker JM, Lieber CS, Black M. Acetaminophen activation by human liver cytochromes P450 IIE1 and P450 IA2. Arch Biochem Biophys 1989;271: 270–283.

79 Roberts JC, Nagasawa HT, Zera RT, Fricke RF, Goon

DJW. Prodrugs of L-cysteine as protective agents against acetaminophen-induced hepatotoxicity. 2-(Polyhydroxyalkyl)- and 2-(polyacetoxyalkyl)thiazolidine-(*R*)-carboxylic acids. J Med Chem 1987;30:1891–1896.

80 Roberts JC, Charyulu RL, Zera RT, Nagasawa HT. Protection against acetaminophen hepatotoxicity by ribose-cysteine (RibCys). Pharmacol Toxicol 1992;70:281–285.

81 Richman PG, Meister A. Regulation of gamma-glutamyl-cysteine synthetase by nonallosteric feedback inhibition by glutathione. J Biol Chem 1975;250:1422–1426.

82 Rumack BH, Peterson RC, Koch GG, Amara IA. Acetaminophen overdose. 662 cases with evaluation of oral acetylcysteine treatment. Arch Intern Med 1981;141:380–385.

83 Sakaida I, Kayano K, Wasaki S, Nagatomi A, Matsumura Y, Okita K. Protection against acetaminophen-induced liver injury in vivo by an iron chelator, deferoxamine. Scand J Gastroenterol 1995;30:61–67.

84 Salminen WF, Voellmy R, Roberts SM. Differential heat shock protein induction by acetaminophen and a nonhepatotoxic regioisomer, 3'-hydroxyacetanilide, in mouse liver. J Pharmacol Exp Ther 1997a;282:1533–1540.

85 Salminen WF Jr, Voellmy R, Roberts SM. Protection against hepatotoxicity by a single dose of amphetamine: the potential role of heat shock protein induction. Toxicol Appl Pharmacol 1997b;147:247–258.

86 Salminen WF, Voellmy R, Roberts SM. Effect of *N*-acetylcysteine on heat shock protein induction by acetaminophen in mouse liver. J Pharmacol Exp Ther 1998;286:519–524.

87 Sarich T, Kalhorn T, Magee S, Sayegh F, Adams S, Slattery J, Goldstein J, et al. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of *S*-mephenytoin. Clin Pharmacol Ther 1997;62:21–28.

88 Sato C, Matsuda Y, Lieber CS. Increased hepatotoxicity of acetaminophen after chronic ethanol consumption in the rat. Gastroenterology 1981a;80:140–148.

89 Sato C, Nakano M, Lieber CS. Prevention of acetaminophen-induced hepatotoxicity by acute ethanol administration in the rat: comparison with carbon tetrachloride-induced hepatotoxicity. J Pharmacol Exp Ther 1981b;218:805–810.

90 Sato C, Lieber CS. Mechanism of the preventive effect of ethanol on acetaminophen-induced hepatoxicity. J Pharmacol Exp Ther 1981c;218:811–815.

91 Schmidt LE, Dalhoff K, Poulsen HE. Acute versus chronic alcohol consumption in acetaminophen-induced hepatotoxicity. Hepatology 2002;35:876–882.

92 Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral *N*-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985). N Engl J Med 1988;319:1557–1562.

93 Smilkstein MJ, Bronstein AC, Linden C, Augenstein WL, Kulig KW, Rumack BH. Acetaminophen overdose: a 48-hour intravenous *N*-acetylcysteine treatment protocol. Ann Emerg Med 1991;20:1058–1063.

94 Smith JA, Hine ID, Beck P, Routledge PA. Paracetamol toxicity: is enzyme induction important? Hum Toxicol 1986;5:383–385.

95 Smith TJ, Hong JY, Wang ZY, Yang CS. How can carcinogenesis be inhibited? Ann N Y Acad Sci 1995;768:82–90.

96 Sohn OS, Fiala ES, Requeijo SP, Weisburger JH, Gonzalez FJ. Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. Cancer Res 2001;61:8435–8440.

97 Srinivasan C, Williams WM, Ray MB, Chen TS. Prevention of acetaminophen-induced liver toxicity by 2(*R,S*)-n-propylthiazolidine-4(*R*)-carboxylic acid in mice. Biochem Pharmacol 2001;61:245–252.

98 Sumioka I, Matsura T, Kasuga S, Itakura Y, Yamada K. Mechanisms of protection by *S*-allylmercaptocystein against acetaminophen-induced liver injury in mice. Jpn J Pharmacol 1998;78:199–207.

99 Sumioka I, Matsura T, Yamada K. Therapeutic effect of *S*-allylmercaptocysteine on acetaminophen-induced liver injury in mice. Eur J Pharmacol. 2001;433:177–185.

100 Sumioka I, Matsura T, Kai M, Yamada K. Potential roles of hepatic heat shock protein 25 and 70i in protection of mice against acetaminophen-induced liver injury. Life Sci 2004;74:2551–2561.

101 Thummel KE, Lee CA, Kunze KL, Nelson SD, Slattery JT. Oxidation of acetaminophen to *N*-acetyl-*p*-aminobenzoquinone imine by human CYP3A4. Biochem Pharmacol 1993;45:1563–1569.

102 Thummel KE, Slattery JT, Ro H, Chien JY, Nelson SD, Lown KE, et al. Ethanol and production of the hepatotoxic metabolite of acetaminophen in healthy adults. Clin Pharmacol Ther 2000;67:591–599.

103 Tonge RP, Kelly EJ, Bruschi SA, Kalhorn T, Eaton DL, Nebert DW, et al. Role of CYP1A2 in the hepatotoxicity of acetaminophen: investigations using Cyp1a2 null mice. Toxicol Appl Pharmacol 1998;153:102–108.

104 Vogt BL, Richie JP Jr. Fasting-induced depletion of glutathione in the aging mouse. Biochem Pharmacol 1993;46:257–263.

105 Wang W, Ballatori N. Endogenous glutathione conjugates: occurrence and biological functions. Pharmacol Rev 1998;50:335–356.

106 Wendel A, Feuerstein S, Konz KH. Acute paracetamol intoxication of starved mice leads to lipid peroxidation in vivo. Biochem Pharmacol 1979;28:2051–2055.

107 Whitcomb DC, Block GD. Association of acetaminophen hepatotoxicity with fasting and ethanol use. JAMA 1994;272:1845–1850.

108 Wlodek L, Rommelspacher H. 2-Methyl-thiazolidine-2,4-dicarboxylic acid protects against paracetamol induced toxicity in human liver derived HepG2 cells. Acta Biochim Pol 1997a;44:759–766.

109 Wlodek L, Rommelspacher H. 2-Methyl-thiazolidine-2,4-dicarboxylic acid as prodrug of L-cysteine. Protection

against paracetamol hepatotoxicity in mice. Fundam Clin Pharmacol 1997b;11:454–459.

110 Yoo JS, Ishizaki H, Yang CS. Roles of cytochrome P450IIE1 in the dealkylation and denitrosation of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine in rat liver microsomes. Carcinogenesis 1990;11:2239–2243.

111 Zaher H, Buters JTM, Ward JM, Bruno MK, Lucas AM, Stern ST, et al. Protection against acetaminophen toxicity in CYP1A2 and CYP2E1 double-null mice. Toxicol Appl Pharmacol 1998;152:193–199.

112 Zhao P, Kalhorn TF, Slattery JT. Selective mitochondrial glutathione depletion by ethanol enhances acetaminophen toxicity in rat liver. Hepatology 2002;36: 326–335.

113 Zhao P, Slattery JT. Effect of ethanol dose and ethanol withdrawal on rat liver mitochondrial glutathione: implication of potentiated acetaminophen toxicity in alcoholics. Drug Metab Dispos 2002;30:1413–1417.

114 Zimmerman HJ, Maddrey WC. Acetaminophen (paracetamol) hepatotoxicity with regular intake of alcohol: analysis of instances of therapeutic misadventure. Hepatology 1995;22:767–773.