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RAPID COMMUNICATION

ACUTE HEPATOTOXICITY OF ACETAMINOPHEN IN RATS TREATED WITH ETHANOL PLUS ISOPENTANOL

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Abstract - Acetaminophen (APAP) hepatotoxicity was investigated in rats fed ethanol and isopentanol alone or in combination in a liquid diet for 7 days. Serum levels of aspartate aminotransferase (AST) and histological examination of liver slices were used to assess hepatotoxicity. At 7 hr after intragastric administration of 0.5 or 1.0 g APAP/kg, there was no significant increase in serum levels of AST in rats treated with APAP alone, or in rats pretreated with ethanol or isopentanol alone followed by APAP. There was mild central lobular congestion in the livers of rats pretreated with ethanol alone followed by APAP. In contrast, in rats pretreated with the combination of ethanol and isopentanol, administration of APAP caused a dramatic increase in serum levels of AST, along with marked central lobular necrosis, including steatosis and ischemic changes. Hepatic glutathione levels were decreased to 40-50% of control values in APAP-treated rats that had been pretreated with ethanol either alone or in combination with isopentanol. The serum concentrations of APAP were significantly lower in rats pretreated with the combination of ethanol and isopentanol followed by 1 g APAP/kg than in rats treated with APAP alone, suggesting a greater rate of APAP metabolism. We had reported previously that combined treatment of rats with ethanol and isopentanol resulted in additive to synergistic increases in CYP3A, with no further increases in CYP2E than that caused by ethanol alone. CYP3A may, therefore, be responsible for the increased APAP hepatotoxicity caused by the combined alcohol treatment.

Key words: acetaminophen; ethanol; isopentanol; Fischer rat; serum transferase; glutathione

APAP‖ is a commonly used analgesic and antipyretic drug. Massive overdose of APAP is associated clinically with severe central lobular hepatic necrosis and death from liver failure [1, 2]. Long-term consumption of alcoholic beverages is associated with development of liver damage and hepatic failure from therapeutic doses of APAP [3, 4]. Hepatotoxicity results from formation of a reactive electrophilic metabolite, NAPQI, by cytochrome P450 [5]. CYP2E1, a major form of P450 increased after consumption of alcoholic beverages in humans [6, 7], is active in the conversion of APAP to NAPQI [8-11]. In experimental animals, long-term treatment with ethanol, the major alcohol in alcoholic beverages [12-14], results in increases in hepatic levels of CYP2E1, as well as increased liver damage from APAP [for review see Ref. 15]. From these findings, CYP2E1

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‖Abbreviations: APAP, acetaminophen; AST, aspartate aminotransferase; CYP, cytochrome P450; GSH, reduced glutathione; and NAPQI, *N*-acetyl-*p*-aminobenzoquinone.

is considered to be the only form of P450 responsible for increased APAP hepatotoxicity associated with consumption of alcohols [for review see Refs. 1 and 15]. Recently, human [9, 10] and rat [9] forms of CYP3A have been shown to convert APAP to NAPQI, with a K_m close to concentrations detected in human serum after administration of therapeutic doses of APAP. We have found that ethanol induces CYP3A in rat hepatocytes in culture [16] and in the liver of intact rats [17], suggesting that CYP3A may have a role in ethanol-mediated increases in APAP hepatotoxicity. In rats, combined treatment with ethanol and isopentanol, the major higher chain alcohol in alcoholic beverages [12-14], results in additive to synergistic increases in hepatic levels of P450 3A1/2, with no further increase in CYP2E1 over that caused by ethanol alone [17]. The purpose of this study was to investigate whether pretreatment of rats with ethanol plus isopentanol would result in greater increases in APAP-mediated hepatotoxicity compared with pretreatment with either alcohol alone. We observed a dramatic increase in hepatic necrosis as well as serum levels of AST in rats pretreated with ethanol plus isopentanol compared with rats pretreated with ethanol or isopentanol alone.

MATERIALS AND METHODS

Chemicals. APAP, isopentanol, and AST and creatinine analysis kits were purchased from the Sigma Chemical Co. (St. Louis, MO). Absolute ethanol (USP) was from the Aaper Alcohol and Chemical Co. (Shelbyville, KY). Methanol and acetonitrile were from Fisher Scientific (Springfield, NJ). The Lieber-DeCarli diet was obtained from the Baker Chemical Co. (Phillipsburg, NJ).

Treatments of rats. Male Fischer 344 rats (280-300 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and maintained in a controlled environment with a 12-hr light/dark cycle. Ethanol (6.3%, w/v) and isopentanol (0.5%, w/v) were administered to the rats alone or in combination for 7 days as part of the Lieber-DeCarli diet, as described [17]. Rats were deprived of food 11 hr before administration of APAP to avoid interference of high blood alcohol levels with the metabolism of APAP [18, 19]. APAP (0.5 or 1.0 g/kg) was administered by intragastric intubation of a supersaturated solution in sterile saline (4 mL). Each dose was individually prepared by adding solid APAP to saline in a syringe barrel, sonicating for 10 sec (Sonifier Cell Disruptor, model W140D) and maintaining at 37° before administration. Seven hours after administration of APAP, rats were anesthetized with CO_2, blood was removed by cardiac puncture, and the animals were decapitated. This protocol was approved by the Institutional Animal Care and Use Committee of the Veterans Administration Medical Center. The livers were excised, and portions of the liver were frozen quickly in dry ice/methanol and stored at -70° for analysis of GSH.
Slices of liver were stored in formalin for histological analysis.

Measurement of serum enzymes. Serum AST and creatinine levels were measured with standard kits according to the manufacturer's instructions.

Measurement of serum APAP levels. Serum levels of APAP were measured by HPLC, as described previously [20], using a Milton Roy CM 4000 multiple solvent delivery system with a Rainin C18 column (5 μm, 4.6 mm x 25 cm). APAP was detected at 254 nm, using an SM-4000 programmable wavelength detector. The mobile phase was 10% acetonitrile, 90% water, and the flow rate was 1.0 mL/min.

Measurement of serum GSH. Hepatic GSH levels were determined by HPLC coupled with electrochemical detection, as described previously [21]. The chromatography was performed isocratically using a Rainin C18 column (5 μm, 4.6 mm x 25 cm) with a mobile phase of 0.1 M monochloroacetic acid, 3.3 mM 1-heptanesulfonic acid (pH 3): methanol (95:5). GSH was detected by electrochemical oxidation (+0.15 V) with a gold-mercury electrode.

Histology. Paraffin sections were prepared after fixation with 10% neutral buffered formalin and stained with hematoxylin and eosin.

Data analysis. Results were analyzed by ANOVA. The Tukey multiple comparison test was used to compare treatment groups and a P value of 0.05 was taken as significant.

RESULTS AND DISCUSSION

Administration of a liquid diet containing ethanol (6.3%, w/v) or isopentanol (0.5%, w/v) or a combination of the two alcohols to rats for 7 days caused no weight loss or other overt signs of toxicity. When these animals were administered APAP (0.5 or 1 g/kg) for 7 hr, there were also no overt signs of toxicity. Serum creatinine levels were unchanged by any treatment (data not shown), indicating no nephrotoxicity. Pretreatment of rats with the combination of ethanol plus isopentanol resulted in dramatic APAP-mediated hepatotoxicity at 7 hr after the administration of APAP. This was indicated both by elevations in serum levels of AST (Table 1) and by histological changes in the liver (Fig. 1). The serum concentrations of AST were elevated to similar levels at the two doses of APAP administered to rats pretreated with ethanol plus isopentanol. At 1.0 g APAP/kg, the histological changes in rats pretreated with the alcohol combination varied from diffuse central lobular congestion with steatosis to severe central lobular necrosis (Fig. 1C, D). Patchy acute inflammation was observed in only one of six rats. At 0.5 g APAP/kg, the histological changes in rats pretreated with the alcohol combination varied from mild central lobular congestion to central lobular necrosis with focal acute inflammation (results not shown). In rats treated with 0.5 g APAP/kg following pretreatment with isopentanol, there were no increases in serum levels of AST (Table 1). In rats administered 1.0 g APAP/kg alone, there were also no significant increases in serum levels of AST (Table 1), although some liver damage was evident. Treatment with 1 g APAP/kg alone resulted in mild focal central lobular congestion in the liver with no evidence of inflammation (Fig. 1A). In rats pretreated with ethanol before the administration of 1 g APAP/kg, serum levels of AST were not elevated significantly (Table 1). In this latter treatment group, some livers appeared normal, whereas in others histological examination revealed a patchy or diffuse central lobular congestion with steatosis and, in some cases, acute inflammation (Fig. 1B).

This is the first report of histological changes in rat livers 7 hr after administration of APAP [19, 22-24]. The early histological damage to the liver that we observed may be due to the strain of rats used. Fischer rats have been reported to be more susceptible to APAP-mediated hepatotoxicity than Sprague-Dawley rats [25].

Table 1. Serum levels of AST

Pretreatment	APAP (g/kg)	AST (units/mL)
Expt. 1		
None (3)	None	200 + 114
None (6)	0.5	404 ± 116
None (6)	1.0	380 ± 46
Ethanol+isopentanol (4)	None	523 ± 393
Ethanol+isopentanol (6)	0.5	2820 ± 874[a]
Ethanol+isopentanol (5)	1.0	2773 ± 1228[a]
Ethanol (4)	None	750 ± 311
Ethanol (6)	1.0	946 ± 462
Expt. 2		
None (6)	None	15 + 3
None (6)	0.5	37 ± 36
Ethanol+isopentanol (8)	0.5	2097 ± 1515[b]
Isopentanol (8)	0.5	61 ± 27

[a]Significantly different from no treatment as well as from treatments with: APAP (0.5 g/kg), APAP (1 g/kg), and ethanol+isopentanol with $P < 0.001$; ethanol and ethanol + APAP (1 g/kg) with $P < 0.01$. [b]Significantly different from no treatment as well as treatment with: APAP (0.5 g/kg), isopentanol + APAP (0.5 g/kg) with $P < 0.001$. Values are means ± SD; the numbers of rats in each group given in parentheses.

The results in Table 2 show that neither ethanol alone nor the combination of ethanol and isopentanol decreased GSH levels in the absence of APAP. APAP decreased hepatic GSH to similar extents (40-50%) in rats

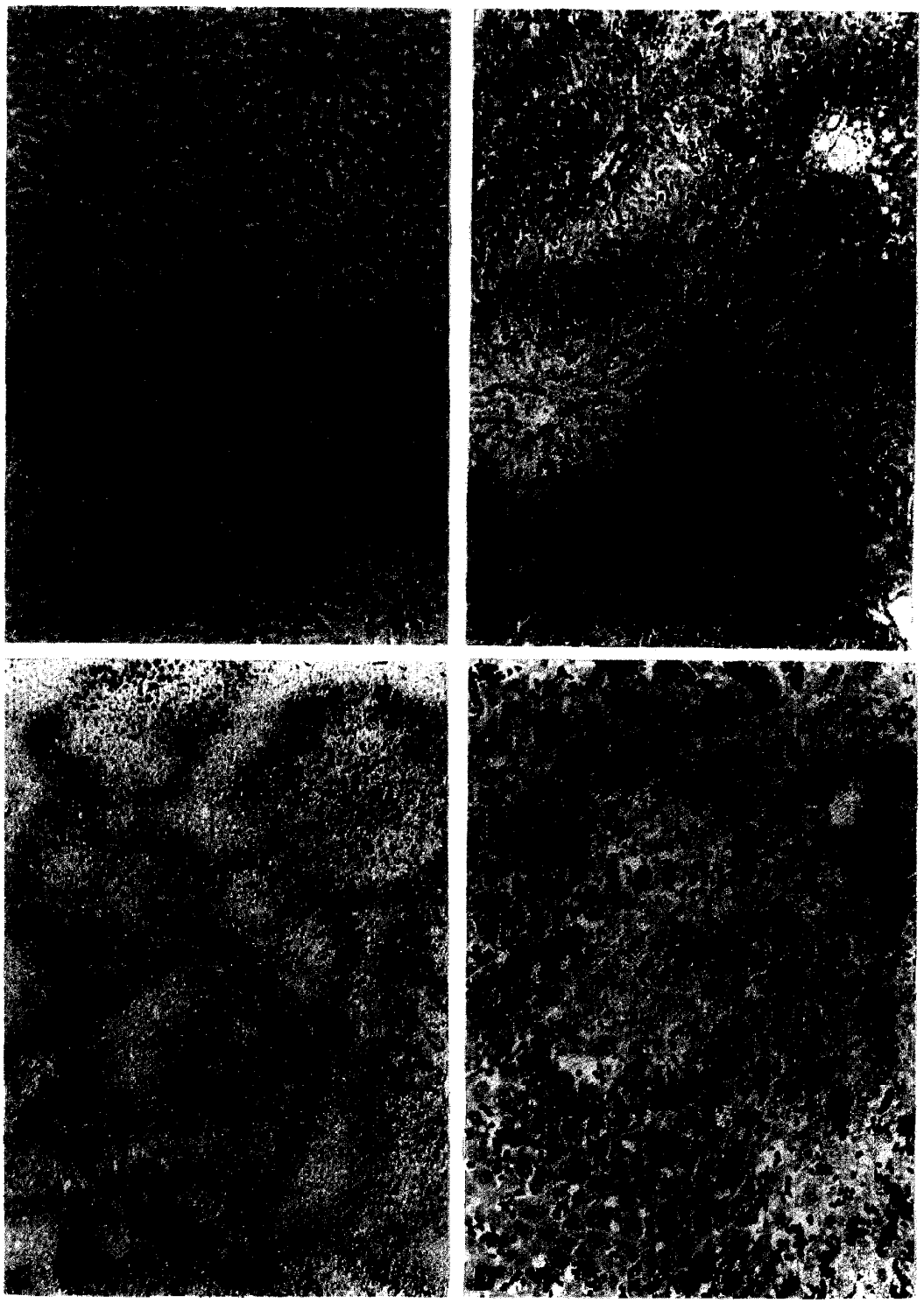

Fig. 1. Histological preparation of rat livers: (A) representative section of rat liver treated with 1 g APAP/kg with mild focal centrilobular congestion (100x); (B) section of rat liver pretreated with ethanol followed by administration of 1 g APAP/kg showing a focus of more prominent centrilobular necrosis (200x); (C and D) representative sections from rats pretreated with ethanol and isopentanol followed by administration of 1 g APAP/kg showing diffuse centrilobular necrosis (100x); (D) close-up reveals karyorrhexis (400x).

pretreated with either ethanol alone or with the combination of alcohols ($P < 0.05$ compared with untreated cells), even though the combined alcohol treatment resulted in greater APAP hepatotoxicity. However, these rats were sacrificed at only 7 hours after the administration of APAP. A greater difference in the hepatic levels of GSH between the two treatment groups may occur at a later time point. Serum concentrations of APAP were lower in rats pretreated with the combination of ethanol and isopentanol before APAP than in rats administered APAP alone (Fig. 2). Thus, decreased serum levels of APAP appear to correlate with hepatotoxicity, as has been reported by others [22, 23], suggesting that toxicity arises from increased metabolism of APAP to NAPQI.

Table 2. Hepatic levels of GSH

Pretreatment	Number of rats	APAP (g/kg)	GSH (μmol/g wet wt)	
None	3	None	3.3 ± 1.4	(100)
None	6	0.5	2.6 ± 0.7	(79)
None	6	1.0	2.3 ± 0.7	(70)
Ethanol+isopentanol	4	None	3.6 ± 1.2	(109)
Ethanol+isopentanol	6	0.5	1.8 ± 0.8*	(54)
Ethanol+isopentanol	5	1.0	1.5 ± 0.6*†	(45)
Ethanol	4	None	3.7 ± 1.0	(112)
Ethanol	6	1.0	1.8 ± 1.0*	(54)

Levels of GSH were measured, as described in Materials and Methods, in the livers of rats from Expt. 1 presented in Table 1. *Significantly different from no treatment with $P < 0.05$. †Significantly different from ethanol+isopentanol alone with $P < 0.05$. Values are means ± SD; numbers in parentheses represent the percent of the mean from untreated rats.

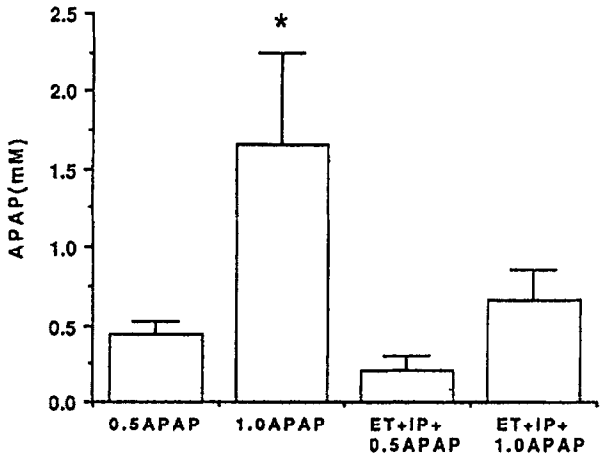

Fig. 2. Concentration of APAP in the serum of rats. Rats were pretreated with alcohols for 7 days. Seven hours after administration of APAP, serum was prepared and the APAP level was measured by HPLC as described in Materials and Methods. ET, ethanol; IP, isopentanol. *Significantly different from: APAP (0.5 g/kg), ET+IP+APAP (0.5 g/kg) with $P < 0.001$ and from ET+IP+APAP (1 g/kg) with $P < 0.01$. Values are means ± SD for five rats.

In summary, we found that APAP hepatotoxicity was greater in rats pretreated with ethanol plus isopentanol than in rats pretreated with ethanol alone. These effects were observed at a concentration of isopentanol in the liquid diet approximating those found in some alcoholic beverages [12-14]. Our results suggest that the isopentanol content of alcoholic beverages may be important in the effect of these beverages on APAP hepatotoxicity. Since we had reported previously that combined treatment of rats with ethanol and isopentanol resulted in additive to synergistic increases in CYP3A [17], our current findings suggest that CYP3A may be responsible for the increased hepatotoxicity from APAP caused by the combined alcohol treatment. The role of CYP2E and CYP3A in development of APAP hepatotoxicity from treatment with ethanol alone and in combination with isopentanol will be examined in future experiments.

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