Acta Medica Okayama

Volume 38, *Issue* 5 1984 *Article* 5

OCTOBER 1984

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Akiharu Watanabe[*] Harushige Nakatsukasa[†]

Michio Kobayashi[‡] Hideo Nagashima[**]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,

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Akiharu Watanabe, Harushige Nakatsukasa, Michio Kobayashi, and Hideo Nagashima

Abstract

Carbon tetrachloride (CCl4)-induced hepatotoxicity was potentiated by pretreatment with beta-phenethyl alcohol, abundantly present in sake. The injury was determined by serum GPT levels and histological examination. Similar results were observed in ethanol- and phenobarbital-pretreated rats. Acetaminophen-induced hepatotoxicity was not accentuated by beta-phenethyl alcohol or ethanol pretreatment. The activities of liver microsomal enzymes, such as cytochrome P-450, cytochrome b5 reductase, aniline hydroxylase and aminopyrine demethylase, were not altered in beta-phenethyl alcohol-pretreated rats. Thus, CCl4-induced hepatotoxicity potentiation by beta-phenethyl alcohol administration is postulated to be due to a mechanism other than increased free radical generation.

KEYWORDS: ?-phenethyl alcohol, ethanol, phenobarbital, carbon tetrachloride, acetaminophen

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*PMID: 6083701 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **38,** (5) 453-459 (1984)

POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY BY β-PHENETHYL ALCOHOL

Akiharu WATANABE, Harushige NAKATSUKASA, Michio KOBAYASHI and Hideo NAGASHIMA

First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan (Director : Prof. H. Nagashima)

Received May 9, 1984

Abstract. Carbon tetrachloride (CCl_4)-induced hepatotoxicity was potentiated by pretreatment with β-phenethyl alcohol, abundantly present in sake. The injury was determined by serum GPT levels and histological examination. Similar results were observed in ethanol- and phenobarbital-pretreated rats. Acetaminophen-induced hepatotoxicity was not accentuated by β-phenethyl alcohol or ethanol pretreatment. The activities of liver microsomal enzymes, such as cytochrome P-450, cytochrome b_5 reductase, aniline hydroxylase and aminopyrine demethylase, were not altered in β-phenethyl alcohol-pretreated rats. Thus, CCl_4-induced hepatotoxicity potentiation by β-phenethyl alcohol administration is postulated to be due to a mechanism other than increased free radical generation.

Key words : β-phenethyl alcohol, ethanol, phenobarbital, carbon tetrachloride, acetaminophen.

Differences in the pathology of alcoholic liver diseases, especially liver fibrosis and alcoholic hepatitis, exist between Japan and Western countries (1). This situation may be related to the consumption of different types of alcoholic beverages. Most popular in Japan is sake, a rice wine. Agents other than ethanol contained in the beverage may be implicated in the induction of liver injury. β-Phenethyl alcohol, an aromatic alcohol, is abundant in sake, at an approximate concentration of 75 mg/l (2), but is scarce in whisky and brandy. Although adverse central nervous system effects are well established, the liver toxicity of β-phenethyl alcohol has not been investigated.

These considerations prompted us to study the different effects of sake and whisky on biochemical and morphological features of liver injury. In the present study, potentiation of carbon tetrachloride (CCl_4)-induced liver damage by β-phenethyl alcohol and other higher alcohols existing in various alcoholic beverages were tested, and the effects of these alcohols were compared to those seen with ethanol and phenobarbital administration.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Awazu Experimental Animal Co., Japan), approximately 200 g each, were maintained on Oriental Laboratory Chow MF and water. A 3-day treatment consisting of daily intragastric administration of one of the following (per kg body weight) was initiated : 4.5 ml of 20 % β-phenethyl alcohol in olive oil, 53 ml of 16 % ethanol in water, 5.9 ml of 20 % N-propanol in water, 17.7 ml of 20 % isoamyl alcohol in olive oil, 7.7 ml of 20 % isobuthanol in olive oil, 53 ml of water and 5.9 ml of olive oil (the last two served as controls). Other rats received intraperitoneal injection of 2.0 ml of 5 % phenobarbital in water or 20 ml of 0.2 % 3-methyl-cholanthrene in olive oil per kg body weight. After finishing the regimens, the rats were given only water until sacrifice. One day after the last administration, 10 ml of 1 % CCl_4 in liquid paraffin per kg body weight or 25 ml of 2 % acetaminophen in water per kg body weight was administered intragastrically or intraperitoneally, respectively. Rats were killed 24 h after the hepatotoxin treatment.

Blood was obtained prior to sacrificing, and serum glutamic pyruvic transaminase (GPT) activity was assayed immediately (3). Liver microsomal fractions were derived from liver homogenates. The contents of cytochrome P-450, cytochrome b_5 reductase, aniline hydroxylase and aminopyrine demethylase were assayed as described previously (4).

Paraffin-embedded specimens were obtained from the anterior portion of the left lateral lobe, stained with hematoxylin and eosin, and examined by light microscopy.

RESULTS

The small dose of CCl_4 produced a slight but significant increases in serum GPT levels in rats pretreated with water and olive oil (Table 1). β-Phenethyl alcohol administration resulted in a significant elevation of serum GPT activity

TABLE 1. EFFECTS OF PRETREATMENT WITH β-PHENETHYL ALCOHOL, OTHER HIGHER ALCOHOLS, ETHANOL AND PHENOBARBITAL ON SERUM GPT ACTIVITIES INDUCED BY CCl_4

Agents	Number of rats	GPT (IU/l)
Water	5	76 ± 12
Olive oil	5	69 ± 13
β-Phenethyl alcohol	8	218 ± 73*
Ethanol	8	580 ± 169*
N-Propanol	6	64 ± 13
Isoamyl alcohol	4	92 ± 23
Isobutanol	3	34 ± 12
Phenobarbital	6	311 ± 65**

Young male rats were given the various agents by gastric intubation daily for 3 days. Phenobarbital was injected intraperitoneally. Twenty-four hours after the last administration, they were treated with a small dose of CCl_4. The values are given as the mean ± standard error of the mean (SEM). The asterisks (*, **) denote $p < 0.05$ or $p < 0.01$ over respective controls (water or olive oil). The serum GPT activity in normal rats was 43 ± 14 IU/l. The administration of phenobarbital, β-phenethyl alcohol, and other alcohols in the absence of CCl_4 did not produce any serum GPT alterations.

after CCl_4 treatment (218 ± 73 IU/l). The other higher alcohols tested did not appreciably alter the serum GPT profiles. Histological examinations demonstrated prominent centrilobular coagulative necrosis in β-phenethyl alcohol-pretreated rats (Fig. 1), with only minor damage elicited by the other agents (data not shown). Preliminary evidence suggests that β-phenethyl alcohol enhancement required a dose of more than 600 mg per kg body weight for more than 2 days' treatment (data not shown). No sex differences were noted.

CCl_4-induced liver injury was also potentiated by ethanol and phenobarbital pretreatments (Table 1). The most extensive injury was noted with ethanol pretreatment. Significant increase ($p<0.05$) in serum bilirubin levels and percent liver weight, (liver weight/body weight) × 100, were observed in ethanol- and phenobarbital-pretreated rats (0.48 ± 0.10 mg/dl, 4.8 ± 0.2 %, and 0.53 ± 0.06 mg/dl, 4.5 ± 0.2 %, respectively), but not in β-phenethyl alcohol- pretreated rats.

TABLE 2. EFFECTS OF PRETREATMENT WITH β-PHENETHYL ALCOHOL, ETHANOL AND PHENOBARBITAL ON SERUM GPT ACTIVITIES INDUCED BY ACETAMINOPHEN.

Agents	Number of rats	GPT (IU/l)
Water	3	22 ± 4
Olive oil	3	61 ± 25
β-Phenethyl alcohol	6	19 ± 1
Ethanol	3	27 ± 6
Phenobarbital	3	1789 ± 892

Young male rats were given β-phenethyl alcohol, ethanol, water or olive oil by gastric tube daily for 3 days. Phenobarbital was injected intraperitoneally. Twenty-four hours after the last administration, they were treated with acetaminophen.

Pretreatments with β-phenethyl alcohol and ethanol before acetaminophen elicit increased serum GPT activity. Serum GPT rose slightly, but significantly, after intraperitoneal administration of acetaminophen to rats pretreated with water and olive oil (Table 2). Acetaminophen-induced liver injury was enhanced by phenobarbital-pretreatment. Serum bilirubin levels and percent liver weight also increased in these rats (data not shown).

No significant alterations in the microsomal drug-metabolizing enzyme activities were observed in β-phenethyl alcohol-pretreated rats. However, phenobarbital and 3-methylcholanthrene produced significant changes (Table 3), as reported previously (5). The increased activities were significant even after accounting for increased liver or body weight.

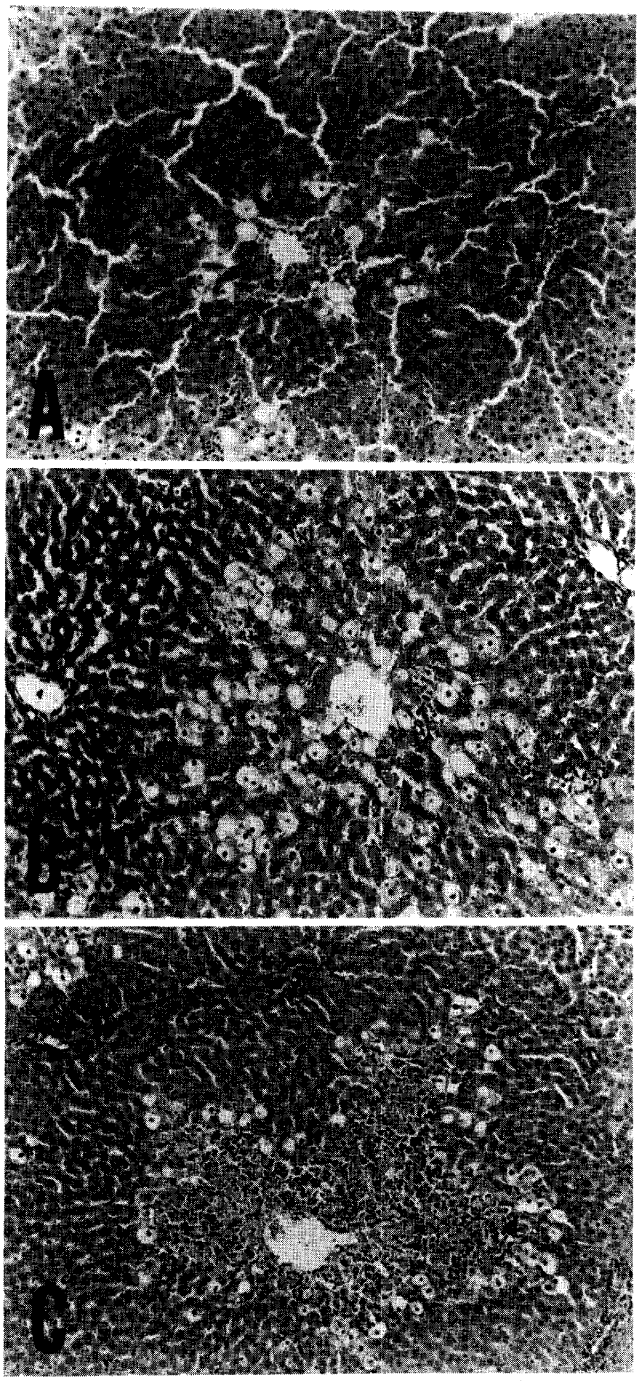
A
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TABLE 3. EFFECTS OF TREATMENT WITH β-PHENETHYL ALCOHOL, ETHANOL, PHENOBARBITAL, AND 3-METHYLCHOLANTHRENE ON ACTIVITIES OF MICROSOMAL DRUG-METABOLIZING ENZYMES

	Water (5)	β-Phenethyl alcohol (7)	Ethanol (6)	Phenobarbital (3)	3-Methyl-cholanthrene (3)
Cytochrome P-450 #	0.73±0.32	0.89±0.20	0.75±0.12	1.90±0.20***	1.27±0.01**
Cytochrome b_5 reductase #	0.30±0.06	0.26±0.12	0.27±0.07	0.27±0.03	0.51±0.02*
Aniline hydroxylase # #	0.77±0.30	0.75±0.33	1.02±0.24	1.46±0.17**	2.14±0.36***
Aminopyrine demethylase # #	6.85±1.64	6.41±1.65	5.97±0.95	12.9±0.60***	6.22±0.88

The asterisks (*, **, ***) denote $p < 0.05$, $p < 0.01$ and $p < 0.001$ against the control (water). Other details are described in Table 1. #, nmoles/mg protein; # #, mU/mg protein. ( ) = Number of rats.

DISCUSSION

Although the reasons for geographic differences in alcoholic liver disease are not clearly understood, the alcoholic vehicle may be involved. In the search for such a constituent, we have been studying β-phenethyl alcohol. Administration of β-phenethyl alcohol potentiated CCl_4-induced, but not acetaminophen-induced liver injury. CCl_4 is activated by the microsomal drug-metabolizing system which generates cytotoxic metabolites and free radicals such as trichloromethyl. Induction of the microsomal enzymes, including cytochrome P-450, by phenobarbital considerably potentiates the toxicity of subsequently administered CCl_4 (6). Unlike CCl_4, acetaminophen is detoxified mainly by glucuronide and sulfate conjugation (7). A small amount of this potential hepatotoxin is activated by the microsomal drug-metabolizing enzymes to a highly toxic metabolite (7). Phenobarbital accentuates the generation of reactive metabolites, thereby enhancing hepatotoxicity, as reported herein. Increased acetaminophen hepatotoxicity has been observed in patients with past histories of chronic ethanol ingestion (7).

A number of alcohols exert potentiating effect on acute CCl_4 hepatotoxicity, isopropanol being a more effective potentiator than ethanol (8). However, the toxicity of thioacetamide and dimethylnitrosamine was found to be potentiated by pretreatment with ethanol but not with isopropanol (9). Although little is known regarding the mechanisms involved in the potentiation, the increased toxicity of CCl_4 after pretreatment with isopropanol has been correlated with increased covalent binding in vivo of $^{14}CCl_4$ to liver lipid and protein (9).

The activities of a number of microsomal enzymes were not elevated by β-

Fig. 1. Effect of pretreatment with β-phenethyl alcohol, ethanol and phenobarbital on the liver histology in CCl_4-intoxicated rats. A) Control (water); B) β-phenethyl alcohol, and C) phenobarbital. In rats pretreated with β-phenethyl alcohol and phenobarbital, centrilobular cell necrosis with inflammatory cell infiltration and hydropic changes were observed, whereas, in water-treated control rats, these changes were minimal. Hematoxylin and eosin, × 250.

phenethyl alcohol or ethanol treatment. Therefore, β-phenethyl alcohol enhancement of CCl_4 toxicity cannot be explained by the invoking of accelerated microsomal metabolism. The lack of an affect on acetaminophen toxicity further argues against this mechanism.

The severity of CCl_4-induced injury can be attenuated by several microsomal inhibitors, but their effectiveness can not be completely explained by inhibition of either CCl_4 metabolism or CCl_4-induced lipid peroxidation *in vitro* (10). An antioxidant, *N*, *N'*-diphenyl-*P*-phenylenediamine, decreases CCl_4-induced liver triglyceride accumulation, not by interaction with the endoplasmic reticulum, but by a non-specific mechanism involving structures unrelated to the primary target of CCl_4 peroxidation (11). Klaassen and Plaa (12) have stated that the molecular basis of CCl_4 hepatotoxicity may not be elucidated fully yet.

Glutathione conjugates reactive toxic metabolites of both CCl_4 and acetaminophen, generating harmless forms such as mercapturic acid (7). CCl_4- and acetaminophen-induced liver injury is accentuated by glutathione depletion (13, 14). If the β-phenethyl alcohol effect was accomplished by decreasing glutathione stores, enhanced acetaminophen toxicity would be expected. This was not seen, suggesting a different mechanism.

These considerations suggest that the mechanism of β-phenethyl alcohol potentiation is not to be found in the microsomal system nor in radical scavenging. It is suggested that CCl_4 hepatotoxicity enhancement is not mediated by β-phenethyl alcohol itself, but by metabolic changes caused by β-phenethyl alcohol administration. Alternatively, a minor, potentially toxic, pathway of CCl_4 metabolism may be accentuated. Although β-phenethyl alcohol did not increase the absolute concentration of microsomal cytochrome P-450, it may affect the levels and activities of minor forms of cytochrome P-450, which function in CCl_4 metabolism. A long-term treatment with phenobarbital results in hepatocyte dysfunction (15) and thus might decrease the susceptibility to CCl_4 hepatotoxicity. Many factors are known to influence CCl_4-induced necrosis (16).

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