Protection of Ethanol-mediated Acetaminophen Hepatotoxicity by Triacetyloleandomycin, a Specific Inhibitor of CYP3A*

VESVOLOD E. KOSTRUBSKY, B.S.,†
JULIANA G. SZAKACS, M.D.,‡§
ELIZABETH H. JEFFERY, M.D.,¶
SHERYL G. WOODS, B.S.,∥
WILLIAM J. BEMENT, B.S.,∥
STEVEN A. WRIGHTON, Ph.D.,‡‡
PETER R. SINCLAIR, Ph.D.,‡‡**
and JACQUELINE F. SINCLAIR, Ph.D.,‡‡**

†Veterans Administration Medical Center, White River Junction, VT 05009
and Departments of ‡Pharmacology/Toxicology, **Biochemistry, and §Pathology, Dartmouth Medical School, Hanover, NH 03756
and ‡‡Department of Drug Disposition, Eli Lilly Research Laboratories, Indianapolis, IN 46285
and ¶Institute for Environmental Studies, University of Illinois, Urbana, IL 61801

ABSTRACT

Cytochrome P450 2E (CYP2E) is considered responsible for ethanol-mediated increases in acetaminophen (APAP) hepatotoxicity. However, it has been shown in cultured human and rat hepatocytes and intact rats that ethanol induces CYP3A in addition to CYP2E. Therefore, an investigation was made in rats to see whether or not an inhibitor of CYP3A, triacetyloleandomycin (TAO), would protect against ethanol-mediated increases in APAP hepatotoxicity. Rats, treated with 6.3 percent ethanol in the Lieber-DeCarli diet for 7 days, were administered APAP (1g/kg, ig) 11 hrs after removal of the diet. Triacetyloleandomycin (500 mg/kg, saline solution) was injected ip 2 hrs before the administration of APAP. In rats pretreated with ethanol, treatment with APAP for 7 hrs resulted in focal centrilobular congestion and

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* Send reprint requests to: Jacqueline F. Sinclair, Ph.D., Research 151, Veterans Administration Medical Center, White River Junction, VT 05009.

‖ Current address: Anatomic Pathology (113), Salt Lake City Veterans Administration Medical Center, 500 Foothill Boulevard, Salt Lake City, UT 84148.
steatosis. Triacetyloleandomycin completely prevented the histological liver damage in all 8 animals. These results suggest that, in ethanol-treated rats, CYP3A plays a major role in increasing APAP hepatotoxicity.

Introduction

In humans, long-term consumption of alcoholic beverages is associated with development of liver damage and hepatic failure from therapeutic doses of acetaminophen (APAP). In experimental animals, long-term treatment with ethanol, the major alcohol in alcoholic beverages, also results in increased liver damage from APAP. Hepatotoxicity is due to cytochrome P450-dependent formation of a reactive electrophilic metabolite, N-acetyl-p-aminobenzoquinone (NAPQI). CYP2E1, a major form of P450 increased after consumption of alcoholic beverages in humans and after exposure of experimental animals to ethanol, is active in the conversion of APAP to NAPQI. Furthermore, CYP2E1 knock-out mice are considerably less sensitive than wild type mice to APAP-mediated liver failure. From these findings, CYP2E1 is considered to be the only form of cytochrome P450 responsible for alcohol-mediated increases in APAP hepatotoxicity.

Ethanol has been shown to increase hepatic levels of CYP3A in several experimental systems including cultured human and rat hepatocytes, a rat hepatoma line and intact rats. Alcohol consumption has recently been shown to increase hepatic levels of CYP3A in humans. Human and rat forms of CYP3A have been shown to convert APAP to NAPQI, with a Km close to concentrations detected in human serum after administration of therapeutic doses of APAP. These findings question whether or not CYP3A has a role in ethanol-mediated increases in APAP hepatotoxicity. An investigation was undertaken to see whether or not triacetyloleandomycin (TAO), a specific inhibitor of CYP3A, would prevent increases in acetaminophen hepatotoxicity in ethanol-pretreated rats.

Methods and Materials

Chemicals

Purchases were made of APAP* and TAO*, absolute ethanol (USP)† and the Lieber-DeCarli diet.§

Treatment of Rats

Male Fischer 344 rats (280 to 300 g) were purchased and maintained in a controlled environment with a 12 hrs light/dark cycle. Ethanol (6.3 percent, w/v) was administered to the rats for 7 days as part of the Lieber-DeCarli diet, as described. The liquid diet was replaced with water for 11 hrs before administration of APAP to avoid interference by high blood alcohol levels with the hepatic metabolism of APAP. The TAO was dissolved in saline (120 mg TAO/ml), adjusted to pH 4, and injected ip at a dose of 500 mg/kg 2 hrs prior to the administration of APAP.

Rats not receiving TAO were administered saline at pH 4. Acetaminophen (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 and maintaining it at 37°C before administration. Seven hours after administration of APAP, rats were anesthetized with CO2 and the animals were decapitated. Slices of liver from all rats were fixed in formalin for histological analysis. These proto-

* Sigma Chemical Co., St. Louis, MO.
† Aaper Alcohol and Chemical Co., Shelbyville, KY.
‡ Baker Chemical Co., Phillipsburg, NJ.
§ Harlan Sprague-Dawley, Indianapolis, IN.
Sonifier Cell Disruptor, model W140D.
columns were approved by the Institutional Animal Care and Use Committee of the Veterans Administration Medical Center.

**HISTOLOGY**

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

**Results**

Examination of all livers from the rats treated with APAP alone revealed normal histology (table I), as was found previously.\(^2^3\) It has been shown previously that treatment with ethanol alone, as part of the liquid diet for 7 days, also caused no histological changes in the liver.\(^1^7,2^3\) However, all rats pretreated with ethanol, prior to administration of APAP, had histological evidence of liver damage. In figure 1A are illustrated moderate centrilobular congestion and microvesicular steatosis in Zone 3, with no involvement of the portal triads, after APAP administration to ethanol-pretreated rats. However, administration of TAO 2 hrs before APAP completely prevented the liver damage, with all 8 animals demonstrating normal histology (figure 1B, table I).

**Discussion**

Previous experiments reporting ethanol-mediated increases in APAP hepatotoxicity in rodents have assumed that CYP2E, which is increased by ethanol, was the only form of cytochrome P450 responsible for the conversion of APAP to the reactive metabolite, NAPQI.\(^1^,3,4,1^3\) However, it has been found in cultured rat\(^1^5\) and human hepatocytes\(^1^6\) and in intact rats\(^1^7\) that ethanol induces CYP3A in addition to CYP2E. To assess the role of CYP3A in ethanol-mediated increases in APAP hepatotoxicity in rats, an investigation was undertaken as to whether or not TAO, a specific inhibitor of CYP3A,\(^2^1,2^2\) would provide protection in these animals.

In these experiments, the TAO was administered in saline instead of dimethyl sulfoxide (DMSO), since DMSO inhibits CYP2E.\(^2^6\) Triacyctyloleandomycin completely prevented APAP-mediated liver damage in ethanol-pretreated rats, as observed histologically (figure 1, table I). The results suggest that CYP3A has a major role in ethanol-mediated increases in APAP hepatotoxicity. This hypothesis is supported by the relative Km values of CYP3A and CYP2E for APAP. The reported Km values of human\(^1^1,2^0\) and rat\(^2^0\) forms of CYP3A range from 0.1 to 0.3 mM, whereas the Km value of CYP2E for APAP is 1 mM.\(^1^1,2^0\) The lower Km of CYP3A for APAP may explain why CYP3A appears to have a major role in liver damage from APAP in ethanol-pretreated rats (figure 1, table I).

This is the first study using TAO, an inhibitor of CYP3A,\(^2^1,2^2\) to assess the role of CYP3A in ethanol-mediated increases in APAP hepatotoxicity. These experiments are now possible
Figure 1. Histological preparation of rat livers. A, Representative section from rats treated with ethanol prior to the administration of APAP (100×) showing centrilobular congestion and microvesicular steatosis with sparing of the portal tract. B, Representative section from rats pretreated with ethanol and administered TAO 2 hrs prior to APAP (100×).
with the recent recognition that TAO specifically inhibits the activity of CYP3A without affecting the activities of other forms of P450, including CYP2E. Although it would be ideal to use a specific inhibitor of CYP2E, several inhibitors which were originally thought to be specific for CYP 2E, such as diethylthiodicarbamate, 4-methylpyrazole, diallylsulfide, and even ethanol itself, have since been proven to inhibit other forms of P450.

In summary, it has been found that TAO, a specific inhibitor of CYP3A, protects ethanol-pretreated rats from developing histologically observable liver damage after exposure to APAP. Recently, consumption of alcoholic beverages was found to be associated with elevated levels of CYP3A in humans, as assessed by the urinary ratios of 6β-hydroxy cortisol to 17-hydroxycortisol. Subsequent abstinence resulted in decreases in CYP3A in these patients. Our results suggest that CYP3A has a major role in alcohol-mediated increases in APAP hepatotoxicity in both experimental animals and humans. Our findings also question whether or not other drugs that induce CYP3A may increase the risk of developing liver damage from acetaminophen in humans.

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References