INFLUENCE OF CEFOPERAZONE ON ETHANOL AND ACETALDEHYDE METABOLISM *IN VITRO*

HEBE B. GREIZERSTEIN

Research Institute on Alcoholism, 1021 Main Street, Buffalo, New York 14203, U.S.A.

ALBERT J. SIEMENS

Pfizer Pharmaceuticals, Inc., 235 East 42nd Street, New York, New York 10017, U.S.A.

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The effect of cefoperazone on ethanol and acetaldehyde metabolism was studied in rat liver homogenates and with a purified aldehyde dehydrogenase. Rat liver homogenates were incubated with ethanol (30 mM) alone or in combination with cefoperazone (15 or 150 μg/g liver). Ethanol and acetaldehyde concentrations were determined at 6, 12, 18 and 24 minutes. Cefoperazone added to the incubation medium inhibited ethanol and acetaldehyde metabolism in a concentration-dependent manner. The addition of cefoperazone to rat liver homogenates incubated with acetaldehyde (300 μM), however, did not inhibit acetaldehyde disappearance for a period of 15 minutes. Purified aldehyde dehydrogenase was incubated with 300 μM acetaldehyde. When cefoperazone was added, acetaldehyde disappearance was significantly slower than without cefoperazone.

The data indicate that cefoperazone inhibits ethanol metabolism in rat liver homogenates in a concentration-dependent manner. The effect of the antibiotic on acetaldehyde elimination in liver homogenate, however, depends on the concentration of acetaldehyde in the medium. The acetaldehyde dehydrogenase obtained from yeast is inhibited by cefoperazone.

The use of alcoholic beverages by subjects receiving cefoperazone, a so-called third generation cephalosporin, has resulted in adverse reactions characterized by flushing, tachycardia, dyspnea, hyperventilation, headache, nausea and vomiting[1,2]. Similar adverse effects have also been observed when ethanol was ingested by subjects receiving other β-lactam antibiotics such as cefamandole[3] and latamoxef (moxalactam)[4]. The symptoms of the cefoperazone-ethanol interaction are analogous to those observed following disulfiram plus ethanol ingestion.

The mechanism of the disulfiram-ethanol reaction is not fully established. However, the primary hypothesis proposes that the response is due to elevated acetaldehyde concentrations in the body which result from an inhibition of aldehyde dehydrogenase by disulfiram[5,6]. Alternatively, the inhibitory effect of disulfiram on dopamine β-hydroxylase could possibly be responsible for the effect[7].

The objectives of this study were to ascertain whether cefoperazone inhibits ethanol and/or acetaldehyde metabolism in rat liver homogenates and to measure the effect of cefoperazone on acetaldehyde degradation by purified aldehyde dehydrogenase.

Methods

Rat Liver Homogenates

Male Wistar rats (Woodlyn Laboratories), 240~260 g, were housed for a week with unlimited access to chow and water. The animals were fasted over night prior to the experiment. On the day of the study, the rats were decapitated, and the livers were immediately excised, weighed and homogenized in 7 volumes of an ice-cold 10 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose, 1 mM EDTA and

0.5 mM NAD^+. Equal volumes (4.5 ml portions) of each liver homogenate (approximately 650 mg liver) were pipetted into each of six incubation flasks (25 ml). All samples were kept at 4°C until the time of incubation. Three of the preparations from each liver were used in the ethanol incubations (Groups 1 ~ 3) and the other three in the acetaldehyde (Groups 4 ~ 6) incubations as described below.

Hepatic Ethanol Metabolism

Ethanol test solutions were prepared in 10 mM phosphate buffer (pH 7.4) and added to incubation flasks in equal volumes (100 μliter) to give the following initial concentrations in the incubation medium: (Solution for Group 1) 30 mM (138 mg%) ethanol; (Group 2) 30 mM ethanol plus 15 μg cefoperazone/g liver; (Group 3) 30 mM ethanol plus 150 μg cefoperazone/g liver.

The incubation flasks, containing liver homogenate and sealed with rubber stoppers, were equilibrated in a Dubnoff shaking incubator at 37°C for 10 minutes. Immediately thereafter, one of the three test solutions was respectively added to each of the three samples from each liver homogenate and equilibration was continued for 2 minutes. At the end of the 2-minute period (designated time zero) and at 6, 12, 18 and 24 minutes thereafter, 500 μliter of the vapor phase was withdrawn from each flask with a gas tight Hamilton syringe and assayed for ethanol and acetaldelyde by gas chromatography.

Hepatic Acetaldehyde Metabolism

Each of the other three samples from each liver homogenate was respectively incubated with one of the following acetaldehyde test solutions; addition of 100 μliter of each solution produced the indicated initial concentrations in the incubation medium: (Solution for Group 4) 300 μM (1.32 mg %) acetaldehyde; (Group 5) 300 μM acetaldehyde plus 15 μg cefoperazone/g liver; (Group 6) 300 μM acetaldehyde plus 150 μg cefoperazone/g liver.

The study conditions were the same as for the ethanol incubations except that vapor samples for assay were taken at 5, 10 and 15 minutes after time zero.

Acetaldehyde Metabolism: Purified Aldehyde Dehydrogenase

A stock solution of the enzyme, aldehyde dehydrogenase from yeast (8 mg protein/250 U), purchased from Boehringer Manheim, was prepared by dissolving 18.75 mg in 150 ml of 10 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose, 1 mM EDTA and 0.5 mM NAD^+. Aliquots (4.5 ml) of the stock solution were incubated with 100 μliter of each of the acetaldehyde test solutions[4~6] as described for the liver homogenates.

Gas Chromatography

Vapor phase samples were analyzed for ethanol and acetaldehyde with a Perkin-Elmer Sigma 1 GC-Data System equipped with a flame ionization detector and a 183 cm-long glass column packed with 20% Carbowax 20 M on Carbopack B. The nitrogen carrier gas flow rate was 18 ml/minute, and the injector, column and detector temperatures were 150, 75 and 180°C, respectively. The retention times were 0.77 minute for acetaldehyde and 2.01 minutes for ethanol.

For each incubation, the chromatographic response for ethanol in the time zero vapor sample was designated to represent 30 mM in the data system. Ethanol concentrations in all subsequent vapor samples were calculated relative to this initial value. Acetaldehyde concentrations were determined by reference to a standard 4.5 ml buffer solution (without liver) containing 300 μM acetaldehyde.

Results

The ethanol and acetaldehyde concentrations obtained during incubation of liver homogenates with ethanol alone and in combination with two doses of cefoperazone are shown in Tables 1 and 2. A two-way analysis of variance, with time and treatment as repeated measures (each liver was studied under 3 treatment conditions — Groups 1 ~ 3), revealed that the ethanol concentration (Table 1) was significantly different among all treatment groups ($F(8,40)=2.33$; $P=0.037$). Comparison of the means, with the error term calculated from all ethanol incubation samples[8], showed significant differences at $P<0.025$ between the group incubated with ethanol alone and the group incubated with ethanol in com-

Table 1. Influence of cefoperazone on ethanol metabolism by liver homogenates.

Treatment	Ethanol concentrations* (mM)				
	0	6 minutes	12 minutes	18 minutes	24 minutes
Ethanol (30 mM)	30.00±0.00	15.21±0.71	13.16±0.35	13.06±0.36	12.61±0.19
Ethanol (30 mM)+ 15 μg cefoperazone/g liver	30.00±0.00	14.92±0.54	13.01±0.44	12.80±0.28	12.53±0.37
Ethanol (30 mM)+ 150 μg cefoperazone/g liver	30.00±0.00	16.53±1.42	14.63±0.51	14.09±0.51	14.40±0.40

* Mean±S.E.M.; N=6/treatment.

Table 2. Influence of cefoperazone on acetaldehyde accumulation in liver homogenates.

Treatment	Acetaldehyde concentrations* (μM)				
	0	6 minutes	12 minutes	18 minutes	24 minutes
Ethanol (30 mM)	26.75±6.01	34.08±6.23	46.05±7.33	66.28±7.39	83.13±5.18
Ethanol (30 mM)+ 15 μg cefoperazone/g liver	22.93±3.24	35.21±4.19	50.60±3.20	73.82±5.42	93.21±7.66
Ethanol (30 mM)+ 150 μg cefoperazone/g liver	23.58±6.05	40.37±8.00	59.82±9.32	80.26±8.34	121.87±16.31

* Mean±S.E.M.; N=6/treatment.

bination with the high concentration of cefoperazone ($F(4,40)=3.21$), and between the two cefoperazone groups ($F(4,40)=3.67$). The groups incubated with ethanol alone or with ethanol in combination with the low concentration of cefoperazone did not differ in ethanol concentrations. These results indicate that cefoperazone inhibited ethanol metabolism in a concentration-dependent manner.

A two-way analysis of variance with time and treatment as repeated measures also showed that acetaldehyde concentrations (Table 2) were significantly different among all three treatments ($F(8,40)=5.13$; $P=0.0002$). The acetaldehyde levels increased with an increase in cefoperazone concentration. Since the ethanol concentrations were affected by the treatments, the observed effects on acetaldehyde levels could theoretically have been a consequence of this variation. Therefore, analysis of covariance with acetaldehyde levels as the variable and ethanol levels as the covariant was carried out to determine the influence of the treatments on acetaldehyde concentrations without the confounding effects of differences in ethanol concentrations. This analysis confirmed that acetaldehyde concentrations were significantly different among the three groups ($F(8,39)=4.52$; $P=0.0006$). The greatest accumulation of acetaldehyde clearly occurred in the presence of the highest concentration of cefoperazone.

The results of the incubation of liver homogenates with 300 μM acetaldehyde alone and in combination with cefoperazone are shown in Table 3. A two-way analysis of variance with time and treatment

Table 3. Influence of cefoperazone on acetaldehyde metabolism by liver homogenates.

Treatment	Acetaldehyde concentrations (μM)*			
	0	5 minutes	10 minutes	15 minutes
Acetaldehyde	300.00±0.00	103.69±7.62	36.09±4.99	20.60±3.14
Acetaldehyde+ 15 μg cefoperazone/g liver	300.00±0.00	75.11±8.75	22.53±5.53	11.53±1.65
Acetaldehyde+ 150 μg cefoperazone/g liver	300.00±0.00	88.76±11.28	24.45±5.36	20.46±2.80

* Mean±S.E.M.; N=6/treatment.

Table 4. Influence of cefoperazone on acetaldehyde metabolism by aldehyde dehydrogenase.

Treatment	Acetaldehyde concentrations (μM)*			
	0	5 minutes	10 minutes	15 minutes
Acetaldehyde	300.00±0.00	73.24±8.63	24.49±2.79	17.58±2.90
Acetaldehyde+ 1.5 μg/ml cefoperazone	300.00±0.00	113.92±10.68	29.75±5.99	18.83±3.58
Acetaldehyde+ 15 μg/ml cefoperazone	300.00±0.00	133.82±15.97	44.48±13.64	26.40±9.01

* Mean±S.E.M.; N=6/treatment.

as repeated measures did not show significant differences among all groups ($F(6,30)=1.91$; $P=0.113$).

The acetaldehyde concentrations after incubation of aldehyde dehydrogenase with acetaldehyde, alone and with cefoperazone, are shown in Table 4. A significant difference ($F(6,45)=3.85$; $P=0.0035$) was observed among the three groups by a two-way analysis of variance with time as a repeated measure and concentration as an independent variable. Cefoperazone inhibited acetaldehyde dehydrogenase in a concentration-related fashion.

Discussion

Cefoperazone inhibited the metabolism of ethanol and its metabolite acetaldehyde in rat liver homogenates in a concentration-dependent manner. These observations are consistent with a previous report[9] that the β-lactam antibiotics, moxalactam, cefamandole, and cefoperazone, each cause an elevation in blood acetaldehyde concentrations when administered (500 mg/kg) to rats 3 or 12 hours before a dose of ethanol (2 g/kg) and suggest a mechanism for the reported ethanol intolerance reactions in cefoperazone-treated patients[1,2]. That is, an elevation in acetaldehyde blood levels in patients following ethanol ingestion could account for the response.

Cefoperazone, however, did not inhibit acetaldehyde disappearance when the aldehyde was added directly to the rat liver homogenate. It has been postulated[10] that the kinetics of acetaldehyde oxidation may be dependent upon the NADH/NAD ratio which is elevated in the presence of ethanol. Although this suggestion requires further study, it may represent one plausible explanation for the apparent difference in the effect of cefoperazone on the hepatic oxidation of acetaldehyde in the presence and absence of ethanol.

In addition, the differential effects on acetaldehyde metabolism in liver homogenates may have been related to the acetaldehyde concentrations in the incubation medium, initially being nonexistent and increasing gradually to 83 μM when ethanol was added as the substrate compared to an initial concentration of 300 μM when acetaldehyde was introduced directly. Aldehyde dehydrogenase activity in rat liver homogenates is present in both the mitochondrial and microsomal fractions,[11~13] but at acetaldehyde concentrations lower than 100 μM, metabolism is primarily a mitochondrial process[14,15]. Thus, it is possible that the mitochondrial enzyme was inhibited by cefoperazone at low acetaldehyde concentrations, whereas the high *Km* microsomal enzyme was not inhibited by the antibiotic at higher acetaldehyde concentrations. Notably, mitochondrial aldehyde dehydrogenase of rat liver is also more sensitive than the cytosol enzyme to disulfiram[13,16]. These observations, together with the finding that cefoperazone inhibited acetaldehyde metabolism by yeast acetaldehyde dehydrogenase when the substrate was added at a high concentration (300 μM), suggests that the effect of cefoperazone is dependent on the character of the aldehyde dehydrogenase.

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