Ethanol as a Xanthine Dehydrogenase Inhibitor

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In the present study, we investigated whether ethanol inhibits the activity of xanthine dehydrogenase. Ethanol and/or inosine were administered to normal subjects, and plasma concentration and urinary excretion of purine bases were measured together with blood concentrations of lactic acid and pyruvic acid. In addition, ethanol and pyrazinamide were administered to these subjects, and plasma concentration and urinary excretion of pyrazinamide and its major metabolites were measured. Increases in plasma concentration and urinary excretion of xanthine induced by a combination of ethanol and inosine were greater than the sums of increases induced separately by ethanol and inosine, although increases in plasma concentration and urinary excretion of uric acid induced by the combination of ethanol and inosine were not different from the sums of increases induced separately by ethanol and inosine. Ethanol increased the ratio of blood lactic acid to blood pyruvic acid and decreased plasma concentration and urinary excretion of 5-hydroxypyrazinamide and 5-hydroxypyrazinoic acid. These results suggest that ethanol inhibits xanthine dehydrogenase presumably by an ethanol-induced increase in the cytosolic concentration of NADH in the liver.

IN PREVIOUS STUDIES,[1-4] it has been demonstrated that ethanol increases purine degradation in humans. These studies have indicated ethanol-induced increases in plasma concentration and urinary excretion of oxypurines (hypoxanthine and xanthine) as important evidence of accelerated purine degradation. In a previous study,[4] we also demonstrated that intake of 0.45 g/kg weight ethanol increased plasma concentration and urinary excretion of oxypurines, but increased neither plasma concentration nor urinary excretion of uric acid, as described by Puig and Fox.[3] In addition, it was demonstrated that ethanol-induced increases in plasma concentration and urinary excretion of xanthine were greater than those of hypoxanthine, being the same as produced by administration of small amounts of allopurinol (xanthine oxidase inhibitor). Recently, Kaneko et al[5] demonstrated that ethanol decreased the conversion of allopurinol to oxypurinol in vivo by an unknown mechanism(s). Since allopurinol is a xanthine oxidase inhibitor and is itself oxidized to oxypurinol by xanthine oxidase and aldehyde oxidase,[6-8] these results suggest that ethanol inhibits the activity of xanthine oxidase, which is present in the form of xanthine dehydrogenase in vivo. Several agents (allopurinol, pyrazinamide, etc.) are oxidized by xanthine dehydrogenase. A decrease in the activity of xanthine dehydrogenase affects the metabolism of these agents, resulting in increases in plasma concentrations of these drugs and/or decreases in plasma concentrations of their oxidized metabolites. Therefore, it is clinically important to identify whether ethanol inhibits the activity of xanthine dehydrogenase. Since inosine is metabolized to hypoxanthine by purine nucleoside phosphorylase and subsequently to xanthine and uric acid by xanthine dehydrogenase, administration of inosine may increase the production of uric acid. On the other hand, pyrazinamide (an antituberculosis drug) is metabolized to pyrazinoic acid by microsomal deamidase and subsequently to 5-hydroxypyrazinoic acid by xanthine dehydrogenase in a classic pathway,[9,10] whereas pyrazinamide is also metabolized to 5-hydroxypyrazinamide by xanthine dehydrogenase[9,11] and aldehyde oxidase[7,8] in an alternate pathway. Therefore, inhibition of the activity of xanthine dehydrogenase decreases the production of 5-hydroxymetabolites of pyrazinamide. In the present study using these substances (inosine and pyrazinamide), we investigated whether xanthine dehydrogenase activity is inhibited in relation to ethanol metabolism.

SUBJECTS AND METHODS

Chemicals

Pyrazinamide and pyrazinoic acid were prepared by Sankyo Pharmaceuticals (Tokyo, Japan). Inosine was purchased from Roussel Morishita (Osaka, Japan). Both 5-hydroxypyrazinamide and 5-hydroxypyrazinoic acid were produced as described previously.[9] NAD and NADH were purchased from Sigma Chemical Co (St Louis, MO). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Subjects and Protocol

Six men (occasional drinkers) aged 32 to 38 years with a body weight between 50 and 61 kg were studied. These subjects had normal results on routine blood chemical analyses, complete blood count, and urinalysis. After informed consent was obtained, two studies were performed as in Fig 1. In brief, in the first study, after an overnight fast, urine was voided completely 1 hour before the intake of ethanol, the first 1-hour urine samples were collected just before the intake of ethanol, and the first blood samples were drawn from the left antecubital vein by heparinized syringes at 30 minutes before the intake of ethanol. After collection of the first urine samples, each subject ingested ethanol (0.8 mL/kg body weight) diluted with water over a 2-minute period. The second urine samples were collected 1 hour after beginning the intake of ethanol, and the second blood samples were drawn at 30 minutes after beginning the intake of ethanol; the third urine samples were collected 1 hour after the second urine collection, and the third blood samples were drawn at 90 minutes after beginning the intake of ethanol. Two weeks later, after an overnight fast, these subjects were administered 60 μmol/kg weight inosine intravenously via the right antecubital vein as a physiologic saline solution containing 2.14 μmol/mL inosine over 1 hour. Urine and blood samples were

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Submitted May 17, 1994; accepted July 23, 1994.

Supported by a grant from the Gout Research Foundation of Japan.

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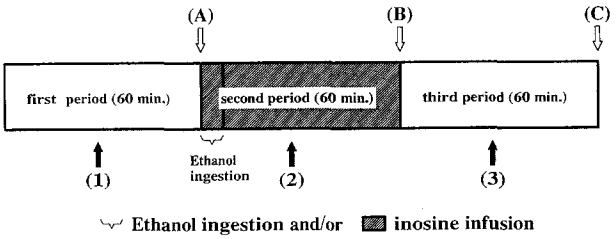

Fig 1. Study protocol. (▨) Inosine infusion; (1) first blood sampling; (2) second blood sampling; (3) third blood sampling; (A) first urine collection; (B) second urine collection; (C) third urine collection. The first study was performed using this protocol, and the second study including administration of pyrazinamide was performed using the same protocol without inosine infusion.

taken before, during, and after administration of inosine at the same times described for the ethanol ingestion. Four weeks later, after an overnight fast, these subjects ingested ethanol (0.8 mL/kg body weight) as described earlier, in addition to being administered 60 μmol/kg weight inosine via the right antecubital vein over 1 hour, and urine and blood samples were taken from the left antecubital vein at the same time as described for the ethanol ingestion.

In the second study, pyrazinamide (0.48 mmol/kg weight) was administered to each subject after a 4-hour fast. Thereafter, after a 10-hour fast, urine was completely voided, the first 1-hour urine samples were collected, and the first blood samples were drawn at 30 minutes before beginning the intake of ethanol. After collection of the first urine samples, these subjects ingested ethanol (0.8 mL/kg body weight) diluted with water over a 2-minute period. The second urine samples were collected 1 hour after beginning the intake of ethanol, and the second blood samples were drawn at 30 minutes after beginning the intake of ethanol; the third urine samples were collected 1 hour after the second urine collection, and the third blood samples were drawn at 90 minutes after beginning the intake of ethanol. In these two studies, water intake was ad libitum, including the fasting periods.

Analysis of Blood and Urine

Plasma and urinary concentrations of uric acid were measured by the uricase method using a Hitachi-736 autoanalyzer (Hitachi, Tokyo, Japan). Plasma concentrations of hypoxanthine, xanthine, and inosine were determined by high-performance liquid chromatography (HPLC). This method is the modified method reported by Yamamoto et al.[12] In brief, fresh heparinized blood was centrifuged for 10 minutes at 1,700 × *g* immediately after withdrawal, and the plasma was taken. Two hundred microliters of plasma was resuspended with 200 μL 5% perchloric acid containing 67.5 μg/mL allopurinol as the internal standard, followed by thorough mixing for 10 seconds. After centrifugation, the clear supernatant was neutralized with 1 mol/L K_2CO_3, and the neutralized supernatant was injected onto a reversed-phase HPLC column (Wakosil 5C18-200, 6 × 250 mm, Wako Pure Chemical Industries). The HPLC system consisted of an SPD-6AV UV-VIS Spectrophotometric Detector, an LC-6A Liquid Chromatograph, an SCL-6B System Controller, a C-R3A Chromatopac (Shimadzu, Kyoto, Japan) and a Wakosil 5C 18-200 column (Wako). Elution conditions were as follows: the mobile phase was 0.02 mol/L KH_2PO_4 (pH 2.2), and the flow rate was 1.0 mL/min. After urine was diluted, urinary concentrations of hypoxanthine and xanthine were also determined by the same method as described for the determination of plasma concentrations of hypoxanthine and xanthine, except for the use of the mobile phase (pH 4.0 instead of pH 2.2). Plasma concentrations of pyrazinamide, 5-hydroxypyrazinoic acid, 5-hydroxypyrazinamide, and pyrazinoic acid were measured using the modified method reported by Yamamoto et al.[13] In brief, plasma-sample treatment was performed as described earlier without addition of the internal standard. Twenty microliters of the neutralized supernatant was injected onto a reversed-phase HPLC column (Wakosil 5C 18-200, 6 × 250 mm). The HPLC system was the same as described earlier. The mobile phase was 0.02 mol/L KH_2PO_4 (pH 2.15), the flow rate was 1 mL/min, and an SPD-6AV UV-VIS Spectrophotometric Detector was used at 254 nm instead of a fluorescence detector, since a UV spectrophotometric detector was more sensitive than a fluorescence detector for the determination of plasma concentrations of pyrazinamide and its metabolites. Urinary concentrations of pyrazinamide and its major metabolites were determined by HPLC as described previously.[14] In brief, urine was diluted fivefold to 10-fold. Twenty microliters of the treated urine sample was injected onto a Wakosil 5C 18-200 column. The HPLC system was the same as described earlier, except with an RF 530 fluorescence HPLC monitor (Shimadzu, Kyoto, Japan) instead of an SPD-6AV UV-VIS Spectrophotometric Detector. The mobile phase was 0.02 mol/L KH_2PO_4 (pH 2.2), and the flow rate was 1 mL/min. A fluorescence detector was used at 410/265 nm. Concentrations of lactic acid, pyruvic acid, acetic acid, and ethanol were determined by enzymatic methods using a lactic acid kit, a pyruvic acid kit (Kiyowa Medix, Tokyo, Japan), a F-kit acetic acid, and a F-kit ethanol (Mannheim, Germany), respectively.

In Vitro Experiment

To investigate the effect of ethanol, acetaldehyde, sodium acetate, or NADH on the activity of xanthine dehydrogenase, an in vitro study was performed as follows, using partially purified human xanthine dehydrogenase. Human xanthine dehydrogenase was partially purified as described previously.[11] In brief, human liver (~100 g) was obtained from a cadaver. It was immediately homogenized with 3 vol 0.25-mol/L sucrose containing 2.5 mmol/L dithiothreitol (DTT) in a polytron tissue homogenizer at 4°C. All subsequent procedures were also performed at 4°C. The homogenate was then centrifuged at 5,000 × *g* for 30 minutes in a Kubota KR-2000T refrigerated centrifuge (Tokyo, Japan). The resultant supernatant was centrifuged at 15,000 × *g* for 90 minutes in a Beckman refrigerated ultracentrifuge (Beckman Instruments, CA). The supernatant (the cytosol fraction) was treated with 30% saturated ammonium sulfate. The suspension was fractionated by centrifugation at 5,000 × *g* for 30 minutes. The resultant supernatant was treated with 50% saturated ammonium sulfate, and then the suspension was fractionated by centrifugation at 5,000 × *g*. The resultant precipitate was resuspended in 30 mL 10-mmol/L phosphate buffer containing 2.5 mmol/L DTT and dialyzed against 200 vol of the same buffer for 24 hours. Three volumes of 10-mmol/L phosphate buffer containing 2.5 mmol/L DTT and 200 mg/mL hydroxyapatite was added to the dialyzed fraction. The suspension was centrifuged at 8,000 × *g* for 10 minutes, and the supernatant was discarded. Precipitated hydroxyapatite was washed three times in succession with 100 mmol/L phosphate buffer containing 2.5 mmol/L DTT, and then 9 vol 300-mmol/L phosphate buffer containing 2.5 mmol/L DTT was added to the precipitated hydroxyapatite. The suspension was centrifuged at 8,000 × *g* for 10 minutes. The supernatant containing xanthine dehydrogenase and xanthine oxidase was concentrated and used in the in vitro study. Thirty micromoles ethanol, 0.1 μmol/L acetaldehyde, 0.8 μmol/L sodium acetate, and 0.15 or 0.08 mmol/L NADH were added to the reaction mixture consisting of 0.3 mL 150-mmol/L potassium phosphate buffer (pH 7.4), 0.3 mL 360-μmol/L xanthine in distilled water, and 30 μL 4.3-mg protein/mL partially purified enzyme (xanthine oxidase + xanthine dehydrogenase) and 0.27 mL 20-mmol/L NAD in distilled water. The reaction was initiated by

addition of xanthine after a reaction mixture was incubated for 5 minutes at 37°C. The activity of xanthine dehydrogenase was then monitored at 340 nm for 10 minutes by a U-3210 spectrophotometer (Hitachi).

Statistical Analysis

Values are presented as the mean ± SD. Statistical significance of differences between values was analyzed using the two-tailed paired *t* test, except for the values of pyrazinamide and its metabolites, which were analyzed using the two-tailed Wilcoxon test.

RESULTS

Effect of Ethanol on Plasma Concentrations of Purine Bases and Inosine and Urinary Excretion of Purine Bases

Plasma concentrations of hypoxanthine and xanthine increased 1.8- and 5.5-fold at 30 minutes and 1.5- and 6.1-fold at 90 minutes after the beginning of ethanol ingestion, respectively, but uric acid did not increase (Table 1). The increase in plasma concentration of xanthine was greater than that of hypoxanthine at 30 and 90 minutes after beginning ethanol ingestion. Plasma concentration of inosine was not changed after ethanol ingestion (Table 1). Urinary excretion of hypoxanthine and xanthine also increased 1.7- and 3.4-fold, respectively, in the 1-hour period after beginning ethanol ingestion (second period) and 1.3- and 2.9-fold, respectively, in the 1-hour period after the second period (third period) as compared with excretion in the 1 hour before beginning ethanol ingestion (first period), but excretion of uric acid did not increase after beginning ethanol ingestion (Table 1). The increase in urinary excretion of xanthine was also greater than that of hypoxanthine after beginning ethanol ingestion (Table 1).

Effect of Inosine on Plasma Concentrations of Purine Bases and Inosine and Urinary Excretion of Purine Bases

Plasma concentrations of hypoxanthine, xanthine, and uric acid increased 15.3-, 2.0-, and 1.1-fold at 30 minutes and 5.8-, 2.4-, and 1.3-fold at 90 minutes after beginning inosine infusion (Table 1). Plasma concentration of inosine also increased 9.6-fold at 30 minutes, although it remained unchanged at 90 minutes. The 1-hour urinary excretion of hypoxanthine increased 22.8-fold in the 1-hour period after beginning inosine infusion (second period) and 9.7-fold in the 1-hour period after the second period (third period) as compared with excretion in the 1-hour period before inosine infusion (first period), and the 1-hour urinary excretion of xanthine also increased 1.4-fold in the second period and 1.6-fold in the third period (Table 1). However, the 1-hour urinary excretion of uric acid did not change in the second and third periods as compared with the first period (Table 1).

Effect of a Combination of Ethanol Ingestion and Inosine Infusion on Plasma Concentrations of Purine Bases and Inosine and Urinary Excretion of Purine Bases

Plasma concentration of hypoxanthine increased 19.0- and 10.4-fold at 30 and 90 minutes after beginning ethanol ingestion + inosine infusion, respectively, and plasma concentration of xanthine increased 16.9- and 18.3-fold at 30 and 90 minutes, respectively, after beginning inosine infusion + ethanol ingestion as compared with the concentration at 30 minutes before beginning inosine infusion + ethanol ingestion (Table 2). Plasma concentration of uric acid remained unchanged at 30 and 90 minutes (Table 2). Plasma concentration of inosine also increased 9.5-fold at 30 minutes after beginning ethanol ingestion + inosine infusion, although it remained unchanged at 90 minutes. Urinary excretion of hypoxanthine and xanthine increased in the 1-hour period after beginning inosine infusion + ethanol ingestion (second period) and in the 1-hour period after the second period (third period) as compared with excretion in the 1-hour period before beginning inosine infusion + ethanol ingestion (first period) (Table 2). The respective increases were 36.6- and 19.5-fold in the second period and 20.8- and 16.8-fold in the third period. However, urinary excretion of uric acid remained unchanged during the study (Table 2).

Comparison Between an Increase in Plasma Concentration and Urinary Excretion of Purine Bases Induced by Ethanol Ingestion + Inosine Infusion and the Sum of Increases Induced by Separate Ethanol Ingestion and Inosine Infusion

The increase in plasma concentration of hypoxanthine, xanthine, or uric acid was calculated by subtracting plasma concentration of hypoxanthine, xanthine, or uric acid at 30 minutes before beginning ethanol ingestion and/or inosine infusion from that of hypoxanthine, xanthine, or uric acid at 30 and 90 minutes after beginning ethanol ingestion and/or

Table 1. Effect of Ethanol Ingestion or Inosine Infusion on Plasma Concentration of Purine Bases and Inosine (μmol/L) and 1-Hour Urinary Excretion of Purine Bases (μmol)

Variables	Ethanol Ingestion			Inosine Infusion		
	1	2	3	1	2	3
Plasma concentration						
Hx	0.60 ± 0.18	1.05 ± 0.19†	0.90 ± 0.33*	0.87 ± 0.41	13.27 ± 5.10†	5.02 ± 0.57†
X	0.40 ± 0.11	2.18 ± 0.67†	2.45 ± 0.94†	0.38 ± 0.21	0.77 ± 0.18*	0.88 ± 0.45†
Ua	350 ± 60	340 ± 60	340 ± 60	330 ± 50	370 ± 60*	420 ± 50†
Hx-R	0.24 ± 0.08	0.3 ± 0.17	0.24 ± 0.08	0.23 ± 0.07	2.20 ± 1.61*	0.30 ± 0.17
1-Hour urinary excretion						
Hx	3.0 ± 0.6	5.0 ± 1.5*	3.9 ± 0.8*	3.7 ± 1.0	84.2 ± 22.5†	35.7 ± 9.2†
X	2.2 ± 0.7	7.5 ± 3.8*	6.3 ± 2.4†	2.6 ± 0.8	3.5 ± 1.0*	4.1 ± 0.7†
Ua	140 ± 20	150 ± 20	130 ± 30	140 ± 30	170 ± 30	200 ± 40

Abbreviations: Hx, hypoxanthine; X, xanthine; Ua, uric acid; Hx-R, inosine; 1, first blood sample; 2, second blood sample; 3, third blood sample.
*$P < .05$, †$P < .01$: *v* respective first periods.

Table 2. Effect of Combination of Ethanol Ingestion and Inosine Infusion on Plasma Concentration (μmol/L) and Urinary Excretion (μmol) of Purine Bases

	Period		
	1	2	3
Plasma concentration			
Hx	0.73 ± 0.21	13.85 ± 4.12†	7.58 ± 5.96*
X	0.43 ± 0.16	7.28 ± 3.89†	7.87 ± 4.00†
Ua	350 ± 40	360 ± 30	390 ± 40
Hx-R	0.26 ± 0.08	2.47 ± 2.06*	0.34 ± 0.21
Urinary excretion			
Hx	3.3 ± 1.0	120.7 ± 50.8†	68.5 ± 42.9*
X	2.5 ± 1.0	48.7 ± 24.7†	41.9 ± 30.1*
Ua	150 ± 30	190 ± 70	200 ± 80

NOTE. Abbreviations are as in Table 1.
*$P < .05$.
†$P < .01$.

inosine infusion, respectively. The increase in urinary excretion of hypoxanthine, xanthine, or uric acid was calculated by subtracting the 1-hour urinary excretion of hypoxanthine, xanthine, or uric acid during the first period from the 1-hour urinary excretion of hypoxanthine, xanthine, or uric acid during the second or third periods. An increase in plasma concentration of hypoxanthine induced by a combination of ethanol ingestion and inosine infusion was not different from the sum of increases induced by separate ethanol ingestion and inosine infusion at 30 and 90 minutes after beginning ethanol ingestion and/or inosine infusion, respectively (Table 3). An increase in urinary excretion of hypoxanthine also was not different from the sum of increases induced by separate ethanol ingestion and inosine infusion in the second period and in the third period (Table 3). In contrast, an increase in plasma concentration of xanthine induced by a combination of ethanol ingestion and inosine infusion was 3.2- and 2.9-fold at 30 and 90 minutes as compared with the sum of increases induced by separate ethanol ingestion and inosine infusion, whereas an increase in urinary excretion of xanthine was 7.5- and 7.0-fold in the second and third periods as compared with the sum of increases induced by separate ethanol ingestion and inosine infusion (Table 3). However, an increase of uric acid induced by a combination of ethanol ingestion and inosine infusion was not different from the sum of increases induced by separate ethanol ingestion and inosine infusion in plasma concentration and urinary excretion at the respective times and periods (Table 3).

Table 3. Comparison Between Increases in Plasma Concentration (μmol/L) and Urinary Excretion (μmol) of Purine Bases Induced by Combination of Ethanol Ingestion and Inosine Infusion and Sums of Increases Induced by Separate Ethanol Ingestion and Inosine Infusion

	Ethanol Ingestion + Inosine Infusion		Separate Ethanol Ingestion and Inosine Infusion	
	Period		Period	
	2 − 1	3 − 1	2 − 1	3 − 1
Plasma concentration				
Hx	13.11 ± 1.68	4.45 ± 0.53	12.40 ± 2.02	3.75 ± 0.56
X	6.85 ± 1.61*	7.43 ± 1.65*	2.15 ± 0.27	2.58 ± 0.29
Ua	10 ± 10	40 ± 20	50 ± 20	90 ± 10
Urinary excretion				
Hx	117.4 ± 20.5	65.2 ± 17.4	82.2 ± 9.2	33.1 ± 3.7
X	46.2 ± 10.1†	39.4 ± 12.1*	6.2 ± 1.7	5.6 ± 1.0
Ua	40 ± 20	50 ± 30	40 ± 20	50 ± 30

*$P < .05$, †$P < .01$: *v* sums of respective increases by separate ethanol ingestion and inosine infusion at the same time as for the combination of ethanol ingestion and inosine infusion.

Concentrations of Ethanol in Blood and Acetic Acid in Plasma

Upon ethanol ingestion, ethanol concentration in blood was negligible at 30 minutes before, 18.3 ± 5.9 μmol/L at 30 minutes, and 15.8 ± 3.5 at 90 minutes after beginning ethanol ingestion. Acetic acid concentration in plasma was negligible at 30 minutes before, 0.63 ± 0.23 μmol/L at 30 minutes, and 0.68 ± 0.12 at 90 minutes after ethanol ingestion. Upon inosine infusion + ethanol ingestion, ethanol concentration was negligible at 30 minutes before, 17.8 ± 4.8 μmol/L at 30 minutes, and 16.3 ± 5.5 at 90 minutes after beginning inosine infusion + ethanol ingestion, and plasma concentration of acetic acid was negligible at 30 minutes before, 0.65 ± 0.18 μmol/L at 30 minutes, and 0.61 ± 0.17 at 90 minutes after beginning inosine infusion + ethanol ingestion. These values were not different at the respective times between ethanol ingestion + inosine infusion and ethanol ingestion. In addition, plasma concentrations of ethanol and acetic acid also were not different at the respective times between ethanol ingestion and ethanol ingestion + pyrazinamide administration (data not shown). Of course, upon inosine infusion, concentrations of ethanol and acetic acid were negligible during the study (data not shown).

Concentrations of Lactic Acid and Pyruvic Acid in Blood

Upon ethanol ingestion or inosine infusion + ethanol ingestion, lactic acid concentration in blood increased at 30 and 90 minutes after beginning ethanol ingestion or inosine infusion + ethanol ingestion as compared with that at 30 minutes before beginning ethanol ingestion or inosine infusion + ethanol ingestion (Table 4). Upon inosine infusion, lactic acid concentration remained unchanged during the study (Table 4). On the other hand, pyruvic acid concentration in blood decreased at 30 and 90 minutes after beginning ethanol ingestion or ethanol ingestion + inosine infusion, although it did not change during inosine infusion (Table 4). Therefore, a ratio of lactic acid to pyruvic acid increased at 30 and 90 minutes after beginning ethanol ingestion or ethanol ingestion + inosine infusion, but it did not change during inosine infusion.

Effect of Ethanol on Plasma Concentration and Urinary Excretion of Pyrazinamide and Its Metabolites

In the control study, plasma concentration and urinary excretion of pyrazinamide and its major metabolites were not changed significantly over 3 hours (Table 5). However,

Table 4. Blood Concentration of Lactic Acid and Pyruvic Acid Upon Ethanol Ingestion, Inosine Infusion, or Combination of Ethanol Ingestion and Inosine Infusion (mmol/L)

	Period		
	1	2	3
Ethanol ingestion			
Lactic acid	0.77 ± 0.17	1.01 ± 0.35*	1.20 ± 0.25†
Pyruvic acid	0.071 ± 0.013	0.036 ± 0.006*	0.031 ± 0.004†
Inosine infusion			
Lactic acid	0.82 ± 0.17	0.75 ± 0.14	0.70 ± 0.17
Pyruvic acid	0.066 ± 0.020	0.056 ± 0.013	0.053 ± 0.011
Ethanol ingestion + inosine infusion			
Lactic acid	0.85 ± 0.18	1.11 ± 0.23*	1.32 ± 0.32†
Pyruvic acid	0.069 ± 0.012	0.039 ± 0.013†	0.039 ± 0.005†

*$P < .05$, †$P < .01$: *v* respective first periods.

plasma concentrations of 5-hydroxypyrazinamide and 5-hydroxypyrazinoic acid decreased 0.8- and 0.7-fold, respectively, at 30 minutes and 0.7- and 0.5-fold at 90 minutes after ethanol ingestion as compared with respective values at 30 minutes before ethanol ingestion (Table 5). Urinary excretion of 5-hydroxypyrazinamide and 5-hydroxypyrazinoic acid decreased 0.8- and 0.7-fold, respectively, during 1 hour after ethanol ingestion (second period) and 0.7- and 0.4-fold, respectively, during 1 hour after the second period (third period) (Table 5). In contrast, plasma concentrations and urinary excretion of pyrazinamide and pyrazinoic acid were not changed significantly (Table 5).

In Vitro Effect of Ethanol, Acetaldehyde, Sodium Acetate, or NADH on the Activity of Xanthine Dehydrogenase

Specific activity of the partially purified xanthine dehydrogenase was 3.8 nmol NADH formed/min/mg protein. Its activity was not inhibited by the addition of either 30 μmol/L ethanol, 0.1 μmol/L acetaldehyde, or 0.8 μmol/L sodium acetate. However, 0.15 and 0.08 mmol/L NADH inhibited the activity 0.13- and 0.49-fold, respectively.

DISCUSSION

The present study demonstrated that intravenous administration of inosine increases plasma concentrations of purine bases and inosine and urinary excretion of oxypurines (Table 1), and it was also demonstrated that ethanol does not increase either plasma concentrations of uric acid and inosine or urinary excretion of uric acid, but increases those of oxypurines, especially xanthine (Table 1), as described in previous studies.[2-4] Orally administered inosine was absorbed in the intestine and converted to uric acid via hypoxanthine and xanthine in the small intestine and the liver, which both possess xanthine dehydrogenase activity, resulting in an increase in plasma concentration of uric acid but negligible increases in oxypurines.[15] Therefore, in the present study, increases in plasma concentration and urinary excretion of hypoxanthine induced by intravenous administration of inosine seem to be mainly ascribable to the degradation of administered inosine in tissues that lack xanthine dehydrogenase activity. In contrast, increases in plasma concentrations of xanthine and uric acid and urinary excretion of xanthine are ascribable to the degradation of administered inosine and its intermediate product (hypoxanthine) in xanthine dehydrogenase–containing tissues (liver and small intestine). Ethanol increased neither plasma concentration nor urinary excretion of uric acid (Table 1), whereas inosine increased plasma concentration of uric acid, although it did not increase urinary excretion of uric acid (Table 1). Nevertheless, ethanol increased plasma concentration and urinary excretion of xanthine more than did inosine (Table 1). Therefore, the ethanol-induced increase in plasma concentration and urinary excretion of xanthine does not seem to be mainly caused by ethanol-induced acceleration of purine degradation. The present results on inosine infusion or ethanol ingestion suggest that ethanol-induced increases in plasma concentration and urinary excretion of xanthine were caused by ethanol metabolism–related inhibition of xanthine dehydrogenase rather than an ethanol-induced increase in purine degradation, although ethanol did not inhibit the activity of xanthine dehydrogenase + xanthine oxidase in vitro. Therefore, to investigate whether ethanol inhibits the activity of xanthine dehydrogenase in vivo, we administered inosine or pyrazinamide, respectively, together with ethanol. As expected, the increase in plasma concentration of xanthine induced by a combination of

Table 5. Effect of Ethanol Ingestion on Plasma Concentration (μmol/L) and Urinary Excretion (mmol) of Pyrazinamide and Its Metabolites

	Ethanol Ingestion			Control		
	Period			Period		
	1	2	3	1	2	3
Plasma concentration						
5-PZA	21.4 ± 5.1	17.2 ± 4.6*	14.7 ± 5.8*	20.5 ± 6.6	20.4 ± 5.9	20.0 ± 5.7
5-PA	9.2 ± 2.5	6.8 ± 2.7*	5.0 ± 2.3*	8.5 ± 2.1	8.7 ± 2.3	8.7 ± 2.7
PA	65 ± 30	66 ± 32	64 ± 30	68 ± 27	64 ± 27	65 ± 26
PZA	217 ± 62	217 ± 68	209 ± 64	205 ± 48	205 ± 52	198 ± 52
Urinary excretion						
5-PZA	0.43 ± 0.13	0.35 ± 0.10*	0.28 ± 0.07*	0.41 ± 0.09	0.40 ± 0.08	0.40 ± 0.10
5-PA	0.34 ± 0.06	0.24 ± 0.04*	0.13 ± 0.04*	0.32 ± 0.06	0.31 ± 0.06	0.31 ± 0.06
PA	0.15 ± 0.05	0.16 ± 0.04	0.14 ± 0.05	0.15 ± 0.05	0.14 ± 0.04	0.15 ± 0.06
PZA	0.02 ± 0.01	0.03 ± 0.03	0.03 ± 0.03	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.02

Abbreviations: 5-PZA, 5-hydroxypyrazinamide; 5-PA, 5-hydroxypyrazinoic acid; PA, pyrazinoic acid; PZA, pyrazinamide.
*$P < .05$ *v* respective first periods.

ethanol and inosine was greater than the sum of increases in plasma concentration of xanthine induced separately by ethanol and inosine (Table 3). Furthermore, the increase in urinary excretion of xanthine induced by the combination was also greater than the sum of increases in urinary excretion of xanthine induced by ethanol and inosine (Table 3), whereas increases in plasma concentration and urinary excretion of uric acid induced by the combination were not different from the sum of increases in plasma concentration of uric acid and the sum of increases in urinary excretion of uric acid induced by ethanol and inosine, respectively (Table 3). These results suggest that xanthine dehydrogenase was inhibited in relation to the metabolism of ethanol. In addition, plasma concentration and urinary excretion of 5-hydroxymetabolites of pyrazinamide (5-hydroxypyrazinamide and 5-hydroxypyrazinoic acid) were decreased by administration of ethanol, but pyrazinamide and pyrazinoic acid were not changed (Table 5). Since pyrazinamide is converted to 5-hydroxypyrazinamide by xanthine dehydrogenase[9,10] or aldehyde oxidase[7,8] and since pyrazinoic acid (a metabolite of pyrazinamide) is converted to 5-hydroxypyrazinoic acid only by xanthine dehydrogenase,[7,9,11] decreases in plasma concentration and urinary excretion of 5-hydroxymetabolites indicated that xanthine dehydrogenase was inhibited in relation to the metabolism of ethanol.

Therefore, the present study suggests that ethanol-induced increases in plasma concentration and urinary excretion of oxypurines are ascribable to a combination of modest ethanol-induced inhibition of xanthine dehydrogenase and increased adenine nucleotide degradation, although in previous studies,[2-4] ethanol-induced increases in plasma concentration and urinary excretion of oxypurines have been recognized as evidence of increased purine degradation in the body. The present further suggests that ethanol-induced inhibition of xanthine dehydrogenase suppressed the ethanol-induced increase in the plasma concentration of uric acid, although the overall effect of ethanol is to increase plasma concentration of uric acid, since ethanol increases adenine nucleotide turnover[2-4] in the body and plasma concentration of lactic acid, inhibiting renal secretion of uric acid.[16]

What inhibited the activity of xanthine dehydrogenase in relation to ethanol metabolism? Ethanol is metabolized to acetaldehyde by alcohol dehydrogenase, being coupled with the conversion of NAD to NADH in the liver. Successively, acetaldehyde is metabolized to acetic acid mainly by aldehyde dehydrogenase, being also coupled with the conversion of NAD to NADH in the liver and extrahepatic tissues. During these reactions, the cytosolic concentration of NAD decreases, that of NADH increases, and the ratio NADH/NAD increases in the body. In fact, an increase in the ratio NADH/NAD in the body was indicated by an increase in the ratio of lactic acid to pyruvic acid in peripheral blood (Table 4), since an increase in the ratio of lactic acid to pyruvic acid in blood reflects an increase in cytosolic redox potential in the body. In the present study, an ethanol-induced increase in the ratio NADH/NAD in liver cytosol was also indicated, since the ratio of lactic acid to pyruvic acid in the hepatic vein is greater than that in the antecubital vein upon administration of ethanol.[17] A previous study[18] demonstrated that rat liver xanthine dehydrogenase and chick liver xanthine dehydrogenase are inhibited by NADH, and the present study also demonstrated that human xanthine dehydrogenase was inhibited by 0.15 and 0.08 mmol/L NADH, which is comparable to the content of NADH in mouse liver (0.22 μmol/g wet weight).[19] Therefore, an ethanol-induced increase in NADH concentration may cause a decrease in xanthine dehydrogenase activity in the liver, resulting in ethanol-induced increases in plasma concentration and urinary excretion of xanthine and ethanol-induced decreases in plasma concentrations and urinary excretion of 5-hydroxypyrazinoic acid and 5-hydroxypyrazinamide. However, further examination is needed to clarify this.

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