Effects of Dietary Ethanol on Ascorbic Acid and Lipid Metabolism, and Liver Drug-Metabolizing Enzymes in Rats

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Summary Effects of dietary ethanol on ascorbic acid and lipid metabolism, and liver drug-metabolizing enzymes in rats fed a semi-purified diet containing a powdered ethanol preparation (30 cal% in the diet) were studied. Administration of ethanol increased urinary ascorbic acid excretion ($p<0.001$) and ascorbic acid level in the liver ($p<0.001$) and the spleen ($p<0.01$). The activity of hepatic aniline hydroxylase was increased ($p<0.05$) by ethanol feeding but that of aminopyrine $N$-demethylase was not. Increases of serum total and high-density-lipoprotein (HDL) cholesterol level, commonly observed by the administration of xenobiotics, were not observed. These results showed ethanol possessed rather similar properties to xenobiotics such as polychlorinated biphenyls (PCB) or 1,1,1-trichloro-2,2-bis($p$-chlorophenyl)ethane (DDT) in some metabolic changes. In this study, no accumulation of lipid in the liver was observed.

Key Words ethanol, ascorbic acid, drug-metabolizing enzymes, cholesterol, liver lipids

Feeding of a diet containing trace amounts of xenobiotics such as PCB, DDT, or pentobarbital causes increases in serum cholesterol, urinary ascorbic acid, and the activity of liver microsomal drug-metabolizing enzymes in rats (1–11). These metabolic responses are greatly influenced by the kinds (1, 2) or the dietary level (3) of xenobiotics and by dietary manipulation, including protein quantity and quality (4–7). The intake of xenobiotics was suggested to increase the requirements of vitamin A (8), ascorbic acid (9–11), and vitamin E (11).

Xenobiotics are fat-soluble and low molecular weight generally. Caffeine is also known to induce microsomal drug-metabolizing enzymes, elevate the serum level of cholesterol, and the urinary excretion of ascorbic acid just like PCB, DDT,

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and other xenobiotics (12, 13). Ethanol, often taken in daily meals, is partially metabolized by cytochrome P450-containing liver microsomal ethanol-oxidizing system, and we supposed that ethanol might have effects on ascorbic acid and cholesterol metabolism and the activity of liver drug-metabolizing enzymes in rats. In this study, we used a powdered ethanol preparation, directly added to the diet.

MATERIALS AND METHODS

Animals and tissue samples. Male Wistar rats, 4 weeks of age, were obtained from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). All the diets and tap water were supplied ad libitum. Room temperature was kept at 22 ± 2°C, and a 12-h light-dark cycle was maintained (0800–2000 h light). Animals were weighed daily. On the last day of the experimental period, diet was removed from the individual cages at 0600 h. The animals were anesthetized with ether and killed at 1300 h within a 60-min period. Blood was collected by heart puncture for serum lipid analysis. Liver was immediately removed, washed in cold saline, wiped off, and weighed for the determinations of the activities of aniline hydroxylase and aminopyrine N-demethylase. The rest of the tissue was stored in a freezer maintained at −20°C until analysis of liver lipids, ascorbic acid, and vitamin A. Spleen was also removed and managed for the analysis of ascorbic acid.

Experiment 1. After being fed a stock diet (CE-2, Nihon Clea Co., Ltd.,

Table 1. Composition of diets.

| Component                     | Amount (%)     |
|-------------------------------|----------------|
| Casein                        | 25.0           |
| 2-Cornstarch                  | 40.2           |
| Sucrose                       | 20.1           |
| Alcock A-300                  | 60.3           |
| Cellulose powder              | 4.0            |
| Salt mixture                  | 5.0            |
| Vitamin mixture               | 0.5            |
| Choline chloride              | 0.2            |
| Corn oil                      | 5.0            |
| Se (as Na₂SeO₃) (mg/kg diet)   | 0.1            |

*Wako Pure Chemical Co., Ltd., Osaka, Japan. b Sato Food Industry Co., Ltd., Komaki, Japan. Composition of Alcock A-300 (g/kg): ethanol, 305; carbohydrate, 650; moisture, less than 50. c This was identical with Harper’s mixture (22). d This was identical with Harper’s mixture (22) except no ascorbic acid is included. Additional vitamins per kg diet: retinyl palmitate, 10,000 IU; ergocalciferol, 4,000 IU. J. Nutr. Sci. Vitaminol.
Tokyo) for several days, rats weighing 70-90 g were divided into control and ethanol groups of six animals each. In this study, we wished to administer as much ethanol as possible without body weight difference between control and ethanol groups. We also wished to feed ethanol as easily as possible. So in experiment 1, initially, 5 w/v% of ethanol solution was administered as drinking water for the first 7 days. For the next 13 days, 10 w/v% of ethanol solution was administered. In these periods, control diet (Table 1) was fed to rats. All the diets and tap water or ethanol solution were supplied ad libitum. No body weight difference between the two groups was observed. In the next period of 43 days, semi-purified diet containing newly developed powdered ethanol Alcock A-300 (Sato Food Industry Co., Ltd., Komaki, Japan) was used. The dietary composition is shown in Table 1. Alcock A-300 contains 30.5 w/w% of ethanol adsorbed in dextrin. Diets and tap water were supplied ad libitum. In this dietary condition, body weight difference was not observed between the two groups. After feeding ethanol, urine was collected for 24 h into 15 ml of 5% metaphosphoric acid, filtered, and frozen for the determination of ascorbic acid excretion. Portions of stored liver and spleen were homogenized with ice-cold 5% metaphosphoric acid. Ascorbic acid in the supernatant and in the urine was determined by the 2,4-dinitrophenylhydrazine method (14). Liver vitamin A was measured in the unsaponifiable matter using the method of Carr-Price reaction (15). Serum was prepared into HDL and other fractions according to the procedure of Ishikawa et al. (16). Total and HDL cholesterol were analyzed by the method of Pearson et al. (17). Serum triglyceride was analyzed by Wahlefeld’s method (18). Liver homogenate was prepared with ice-cold 1.15% KCl in 0.01 M phosphate buffer, pH 7.4, with a Potter-Elvehjem homogenizer. The crude homogenate was spun at 10,000 x g for 10 min. The postmitochondrial supernatant was used for assays of aniline hydroxylase and aminopyrine N-demethylase, which were described elsewhere (1, 5). Total liver lipids were extracted by the method of Folch et al. (19) and determined gravimetrically. Liver cholesterol was analyzed by the method of Pearson et al. (17). The total lipid phosphorus was measured by the method of Bartlett (20) and the values were multiplied by 25 to obtain the phospholipid content. Liver triglyceride was calculated as the difference between the value of total lipids and that of cholesterol plus phospholipid.

Experiment 2. This was conducted essentially the same as experiment 1, except that the diet containing powdered ethanol was fed ad libitum to rats from the start of the experiment. Feeding period was 26 days. Tissue ascorbic acid, liver enzyme activities, serum lipids, and liver lipids were determined according to methods described for experiment 1.

Statistical analysis. The data were analyzed statistically by Student’s t-test (21).
RESULTS

No effect of ethanol on the growth of rats was observed in both of the experiments. Liver weights were also not different. Figure 1 shows the urinary ascorbic acid excretion in experiment 1. On day 14–15, ethanol was administered in drinking water; the excretion began to increase, reached peak level on day 23–24, and decreased from day 42–43. These was significant difference ($p < 0.001$) in each point on and after day 14–15. Liver and spleen ascorbic acid in rats fed ethanol were higher than in controls ($p < 0.001$, liver; $p < 0.001$, spleen). Liver vitamin A in ethanol group was lower than that in the control group in experiment 1 but no significant difference was observed in experiment 2, wherein the experimental period was shorter (Table 2).

Table 2. Effects of dietary ethanol on tissue ascorbic acid and liver vitamin A in rats.*

| Group     | Expt. period (days) | Ascorbic acid | Liver vitamin A (IU/g tissue) |
|-----------|---------------------|---------------|-------------------------------|
|           |                     | Liver (µg/g tissue) | Spleen (µg/g tissue) | |
| Expt. 1   | 63                  | 253±15         | 422±21                       | 1072±33 |
| Control   | 63                  | 345±5**        | 518±13*                      | 889±57* |
| Ethanol   | 63                  |                |                               |        |
| Expt. 2   | 26                  | 256±18         | 375±7                        | 760±58  |
| Control   | 26                  | 342±12**       | 440±20**                     | 749±28  |
| Ethanol   | 26                  |                |                               |        |

*Initial body weight was 70–90 g. Values are means±SEM ($n=6$). *Significantly different from each of control ($p<0.01$). **Significantly different from each of control ($p<0.001$).
Activity of liver aniline hydroxylase was increased by ethanol but that of aminopyrine N-demethylase was unaffected (Table 3).

Serum lipids are presented in Table 4. The increases of both serum total and HDL cholesterol, which are observed by administration of xenobiotics ordinarily, were not observed. But the level of triglyceride was 1.5-fold higher in rats fed ethanol than in control rats.

No accumulation of lipids in the liver was induced in this experimental condition in which ethanol was fed directly mixed in a semi-purified diet. The levels of lipid fractions, cholesterol, phospholipid, and triglyceride, were not significantly different between each group (Table 5).

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Table 5. Effect of dietary ethanol on liver lipids in rats.*

| Group    | Expt. period (days) | Total lipids  | CHL  | PL  | TG  |
|----------|---------------------|---------------|------|-----|-----|
|          |                     | (mg/g liver)  |      |     |     |
| Expt. 1  |                     |               |      |     |     |
| Control  | 63                  | 45.2±1.9      | 2.50±0.04 | 27.2±0.8 | 15.6±2.1 |
| Ethanol  | 63                  | 49.1±0.9      | 2.39±0.07 | 30.6±0.4 | 16.1±1.1 |
| Expt. 2  |                     |               |      |     |     |
| Control  | 26                  | 52.1±1.7      | 3.49±0.09 | 32.0±0.5 | 16.6±1.9 |
| Ethanol  | 26                  | 45.9±1.2      | 3.25±0.17 | 30.2±0.4 | 12.7±1.1 |

*Values are means±SEM (n=6). Abbreviations used: CHL, cholesterol; PL, phospholipid; TG, triglyceride.

DISCUSSION

The most striking effect of ethanol was on ascorbic acid metabolism in this study (Fig. 1, Table 2). Horio et al. (23, 24) demonstrated that the hepatic synthesis of ascorbic acid was accelerated in rats exposed to several xenobiotics. The acceleration of hepatic synthesis of ascorbic acid was thought to be the reason for increased excretion of urinary ascorbic acid. It was suggested that the feeding of xenobiotics might increase the requirement of ascorbic acid in guinea pigs (9–11). Present data might reflect the acceleration of the synthesis of ascorbic acid in rats fed ethanol. Lester et al. (25) recommended more ascorbic acid intake for heavy drinkers. The decrease in hepatic vitamin A is also observed by the exposure of xenobiotics. An elevated requirement of vitamin A was suggested in rats exposed to xenobiotics (8). So, the change of vitamin A and C requirements should be considered when a large quantity of chronic ethanol as well as xenobiotics is administered.

Rubin et al. (26) and Khanna et al. (27) reported that chronic ethanol treatment induced liver drug-metabolizing enzymes and caused the change of drug metabolism. Lieber et al. (28, 29) clarified that microsomal ethanol-oxidizing system is induced by chronic ethanol treatment. In our results, only aniline hydroxylase activity was elevated and aminopyrine N-demethylase activity was not. PCB or DDT markedly increased both enzyme activities (1, 3). The effect of ethanol on these enzyme activities might be weak.

In many previous reports, the exposure to xenobiotics increased the serum total and especially HDL cholesterol levels (1–8, 10–13, 30–34). The hepatic choledrogenesis was promoted (30, 35, 36). On the other hand, chronic ethanol administration raised the serum cholesterol level (26, 37, 38). The hepatic choledrogenesis was also promoted (37). No effects of chronic ethanol on cholesterol metabolism were found in our present study. (Tables 4 and 5).
alterations of lipid metabolism were considered to be widely affected by dietary content of ethanol and/or fat (39–43). The dietary condition in the present experiments might not be able to affect the cholesterol metabolism. We are going to refer to this point later.

The effects of ethanol on ascorbic acid, liver drug-metabolizing enzymes, and serum cholesterol were slight even though there was a relatively large quantity of chronic ethanol treatment to rats. We guessed that ethanol might have slight effect, like xenobiotics on rats, and the metabolic change of ascorbic acid was significant.

The method of chronic ethanol administration has been devised because ethanol is a liquid. The addition of ethanol into drinking water is very easy, but large-quantity administration is difficult (39). Lieber et al. (39, 40, 43) developed a liquid diet and it is widely used in many experiments concerning ethanol metabolism. In our present study, we fed ethanol to rats in a semi-purified diet by using newly developed powdered ethanol “Alcock A-300.” Alcock A-300 contains about 30.5% of ethanol adsorbed in dextrin. It can be mixed easily with other dietary ingredients and can be conveniently fed to the animals. Chronic ethanol administration in a liquid diet induced the lipid accumulation in the liver and the increase of serum triglyceride and cholesterol (39–41). But our diet did not induce the fatty liver but increase the serum level of triglyceride (Tables 4 and 5). The dietary contents of ethanol and/or fat widely affect the lipid accumulation in the liver. Lieber et al. (42) reported that fatty liver was induced when the dietary content of ethanol was 36 energy% using liquid diet and that the accumulation of triglyceride in the liver was difficult when the dietary fat content was less than 25 energy%. We, in this study, used a dietary condition similar to those of our previous studies for investigating effects of xenobiotics (1–11, 13). So, ethanol content in our diet was about 30 energy% and that of fat was about 10 energy%. The ethanol content in our diet was nearly equal to that of Lieber et al. (39, 40), but fat content seemed to be low. This difference might be one of the factors which could account for non-accumulation of liver lipids in our rats. But powdered ethanol might be absorbed and exert lipid metabolism in rats because serum triglyceride was increased. Powdered ethanol is useful as a dietary ingredient for investigating the metabolic changes in animals.

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