Lowering and Delaying Actions of Bovine Bile on Plasma Ethanol Levels in Rats

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Summary The effects of bovine bile (50–400 mg·kg\(^{-1}\) BW) on plasma ethanol levels in male rats (6–8 weeks old) were examined. Bovine bile decreased and delayed the peak of plasma ethanol concentration: a dose response to bovine bile was observed in the concentration and time to maximum concentration of ethanol but no change in disappearance rate. These phenomena were observed in two conditions: 1) oral administration of bovine bile before oral intubation of ethanol (1.0 g·kg\(^{-1}\) BW) and 2) simultaneous oral administration of bovine bile and ethanol. Similar responses were obtained in taurocholic acid. No changes in hepatic alcohol and aldehyde (low \(K_m\) and high \(K_m\)) dehydrogenase activities were observed. The remaining rate of ethanol in stomach was significantly higher with administration of bovine bile. A negative correlation between the maximum ethanol concentration and the remaining rate of ethanol in stomach was found. The intestinal absorption rate of ethanol decreased significantly in the presence of bovine bile. These results suggest that the delay of the gastric emptying and/or the decrease of the intestinal absorption rate of ethanol are major mechanisms for the decreasing and delaying effects on plasma ethanol by bovine bile. The present paper also suggests that bile acids such as taurocholic acid may participate in the lowering and delaying actions on the peak of plasma ethanol concentration by bovine bile.

Key Words bovine bile, bile acid, ethanol metabolism, gastric emptying, absorption of ethanol, rats, alcohol dehydrogenase, aldehyde dehydrogenase, ethanol

It has been known for a long time that the rate of ethanol (ethyl alcohol) metabolism in human or experimental animals may be modified by amino acids, organic acids and so on (1, 2). For instance, it has been reported that a significant decrease of blood ethanol level induced by glutamine or alanine is due to the acceleration of the ethanol metabolism in the liver by decreasing the ratio of NAD
to NADH (1,3) as a result of activation of mitochondrial electron transport by ADP following ethanol treatment (4). When ethanol and sodium acetate were orally administered simultaneously to rats, blood ethanol level significantly decreased (5). Further, organic acids such as acetic acid, citric acid and vinegars decreased blood ethanol concentration (6). The possible cause of this effect was due, at least in part, to the decreased rate of gastric emptying of ethanol, without changing ethanol metabolizing activities (7). The precise mechanism of the blood ethanol decreasing effects of organic acids, however, it still uncertain.

The factors which control the absorption, distribution and metabolism of ethanol in vivo are unclear (8). To obtain the complete elucidation of the variation mechanisms of ethanol metabolism in vivo, it is essential to investigate the factors which control blood ethanol concentration. However, little is known of the underlying mechanism by which many factors change ethanol metabolism in either human or experimental animals. For intrinsic understanding of this purpose, it is crucial to investigate: 1) the change of the absorption rate of ethanol through gastrointestinal tract, 2) the change of the rate of ethanol metabolism in the liver, 3) the change of ethanol clearance in the body or 4) the change of the rate of the excretion of ethanol from urine and sweat glands.

The hepatic alcohol dehydrogenase [ADH: EC 1.1.1.1] and the hepatic aldehyde dehydrogenase [ALDH: EC 1.2.1.3] are considered to play an important role in the biosynthesis of bile acids from cholesterol (9). Moreover, 5β-cholestan-3α,7α,12α,26-tetrol: NAD+ 26-oxidoreductase in rat liver cytosol and 3α,7α,12α-trihydroxy-5β-cholestane-26-aldehyde in the horse liver mitochondria seem to be identical with ADH and ALDH, respectively (10, 11). This clearly indicates that ADH and ALDH can catalyze an essential reaction in the normal biosynthesis of bile acids. However, effects of bile on ethanol metabolism are still unknown.

The unknown problems are of critical importance to the understanding of 1) the magnitude of the acute intoxication effect of ethanol, 2) the pharmacological and physiological actions of bile, and 3) the interaction of bile with ethanol in the gastrointestinal tract (5). Therefore, it is of critical importance for better understanding of the linkage between bile and ethanol metabolism to investigate effects of the bile acids on ethanol metabolism.

This paper describes, thus, 1) effects of timing of the administration of bovine bile on the plasma ethanol concentration, 2) effects of bovine bile and taurocholic acid on the time course of plasma ethanol concentration in rats, 3) effects of bovine bile on ADH and ALDH activities in the rat liver, 4) effects of bovine bile on the gastric emptying rate of ethanol, and 5) effect of bovine bile on the absorption rate of ethanol in the small intestine.

**METHODS**

**Animals.** Male Sprague-Dawley rats (6 weeks old: 134±0.4 g (n = 104) and 8 weeks old: 255±2 g (n = 56), in mean±SE; CLEA Japan, Tokyo) were used.
The animals were normally housed in the cage maintained at an air temperature of 23±1°C and at the relative humidity of 55±5%. Experimental animals were maintained according to recognized standards to safeguard their welfare. Lighting was controlled automatically for 8:00-20:00. Animal diet (MF-type, Oriental Yeast Co., Tokyo) and once-boiled water were given to the rats ad libitum. The rats were deprived of diet for 16 h and water for 1 h before the experiment.

**Preparation of bovine bile.** Fresh bile was collected from the gallbladder in male bovine. The bovine bile was treated for 5 min at 80°C and centrifuged at 3,000 rpm for 5 min. The supernatant fraction was lyophilized in the presence of 1% silicone for 24 h.

**Effects of the timing of oral administration of bovine bile on plasma ethanol level.** The following three conditions were employed: 1) oral administration of the bovine bile (0.4 g·kg⁻¹) 30 min before oral intubation of ethanol (1.0 g·kg⁻¹, as 20%), 2) simultaneous oral administration of bovine bile (0.4 g·kg⁻¹) and ethanol (1.0 g·kg⁻¹), and 3) oral administration of bovine bile (0.4 g·kg⁻¹) 30 min after oral intubation of ethanol (1.0 g·kg⁻¹). In the control rats, 0.9% NaCl solution instead of the bovine bile solution was orally given with the solution volume being equal, via a stomach tube to the rats.

We also determined the time course of plasma ethanol concentration when ethanol (1.0 g·kg⁻¹) was intraperitoneally administered 30 min after oral intubation of bovine bile (0.4 g·kg⁻¹) to the rat. After the oral intubation or intraperitoneal injection of ethanol, plasma ethanol concentrations in rats were assayed at constant interval.

**Dose effect of bovine bile or taurocholic acid to plasma ethanol level.** Solutions of 2, 4, 8 or 16% were prepared by diluting bovine bile with distilled water. The osmotic pressure of their bile solutions was adjusted to ca. 310–315 mOsm·kg⁻¹ H₂O with 1 M NaCl solution. The volume of administration of bile solution to rats was constant, 1/400 of body weight. Rats received 50, 100, 200 and 400 mg·kg⁻¹ by intragastric intubation with 2, 4, 8 and 16% bovine bile solution, respectively. Thirty minutes after intragastric intubation, ethanol (1.0 g·kg⁻¹) was administered orally via a stomach tube to the rats. The room temperature was maintained at 25 ±1°C. In the control rats, an equivalent volume of 0.9% NaCl solution was given instead of bovine bile in the same manner. For in vivo experiments of the acute oral administration of bile acid to rats, 50 or 200 mg·kg⁻¹ of taurocholic acid was adopted.

**Analysis of plasma ethanol concentration.** After the administration of ethanol to rats, blood samples (~25 µl) were collected with heparinized microcapillary tubes from the tail vein, at constant intervals, and centrifuged promptly at 10,000 rpm for 3 min at 2°C. During experiments, we performed with the least possible pain or discomfort to the animals. The supernatant fraction was used as the sample for measuring ethanol concentration. The plasma ethanol concentrations were analyzed by a gas chromatograph (model 163, Hitachi Co., Tokyo) with a flame ionization detector. The analytical conditions were as follows: column packing,
PEG-20M (Gasukuro Kogyo Co., Tokyo); stainless steel column, $\phi$(i.d.) = 2 mm, $l=200$ cm; column temperature, 80°C; flow rate of nitrogen gas, 25 ml·min$^{-1}$; flow rate of hydrogen gas, 25 ml·min$^{-1}$; air pressure, 0.5 kg·cm$^{-2}$; injection temperature of sample, 90°C; and injection volume of sample, 1 $\mu$l.

**Analysis of the time course of plasma ethanol concentration.** To analyze quantitatively the time course of plasma ethanol concentration, we introduced the following four empirical parameters: maximum plasma ethanol concentration, $C_{\text{max}}$; the time to reach $C_{\text{max}}$, $T_{\text{max}}$; disappearance time of plasma ethanol, $T_{\text{disap}}$; and the apparent first order rate constant of plasma ethanol elimination process, $k_{(\text{app})}$.

**Analysis of ethanol concentration in stomach.** The animals were decapitated 240-270 min after ethanol intubations and then the abdomen of the rat was incised rapidly with scissors. After the cardia and pylorus of stomach were rapidly ligated with the suture, the stomach was isolated promptly and the total ethanol content in stomach was weighed. The fluid contents in the stomach were carefully collected with a microcapillary tube. The concentrations of ethanol in the sample were determined with a gas chromatograph as already described.

**Assays of liver cytosolic ADH and mitochondrial ALDH (high $K_m$ and low $K_m$) activities.** Enzyme activities were determined spectrophotometrically; ADH at 37°C and pH 9.0 with ethanol as substrate (12) and ALDH as described earlier (13). In ALDH assay, high $K_m$ isozyme activity was calculated by subtracting low $K_m$ enzyme activity from the combined activity. Protein contents of the subcellular fractions were determined by the method of Lowry et al. (14).

**Measurements of gastric emptying.** The remaining rates of ethanol in stomach were analyzed to clarify whether the change of gastric emptying was involved in the effects of bovine bile on plasma ethanol levels. The bovine bile (0.4 g·kg$^{-1}$) was administered to rats orally. Thirty minutes after the intubation, ethanol (1.0 g·kg$^{-1}$) was administered orally. In the control group, 0.9% NaCl solution of an equivalent volume was administered orally to rats. The rats were decapitated 10 or 30 min after intubation of ethanol. The stomach of the rat was excised promptly with ligations at the cardia and the pylorus. After weighing the stomach with contents, ethanol concentration in stomach was analyzed with a gas chromatograph as described already. The samples for analyzing plasma ethanol concentration were collected via a tail vein, 1 min before decapitation. The remaining rate of ethanol in stomach was calculated using the following equation: $(=(\text{content of ethanol in stomach/total content of ethanol administered})\times 100)$. 

**Measurement of in situ intestinal absorption rate of ethanol.** To clarify the effect of bovine bile on the rate of absorption of ethanol through the small intestinal membrane, we measured the absorption rate of ethanol in the presence or absence of bovine bile for 5 min at 37°C. The measurements were carried out essentially according to the method of Levine and Pelikan (15). The animals were anesthetized intraperitoneally with 40 mg·kg$^{-1}$ of pentobarbital sodium (as 20% Nembutal solution). After the abdomen had been opened, the small intestine was exposed and the proximal ligature of the loop placed 3 cm from the pylorus. The loop was 7 cm
The loop was washed with 2 ml of 0.9% NaCl solution for two times. The bovine bile (100 mg) solution containing 100 mg of ethanol was then injected into the loop. In the control experiment, 0.9% NaCl solution containing 100 mg of ethanol was injected into the loop instead of the bovine bile solution. The volume of solution injected into the loop was 0.5 ml. The absorption experiment was carried out for 5 min and then the loop was isolated quickly. Further, the content in the loop was collected and weighed promptly. Ethanol concentration in the loop was analyzed with a gas chromatograph as described already. The rate of absorption of ethanol through the small intestine was presented as mg of ethanol per min per cm loop.

Analysis of bile acids in bovine bile. The analysis of bile acid in bovine bile was carried out using the method of Hayashi et al. (16).

Statistical analysis. Data were expressed as mean ± SE. The statistical significance of the data was evaluated by Student's t-test for unpaired comparison or Dunnett's test. Differences were considered significant if p was < 0.05.

RESULTS

Effects of the timing of oral administration of bovine bile on plasma ethanol level

Figure 1A shows the time courses of the plasma ethanol concentration when bovine bile (0.4 g·kg⁻¹) 30 min before oral intubation of ethanol (1.0 g·kg⁻¹) was

![Graph showing the effects of bovine bile on plasma ethanol level under various conditions.](image)

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administered orally. In this condition, the bovine bile decreased and delayed the peak of plasma ethanol concentration. After 240 min of ethanol administration, the contents of taurocholic acid of bovine bile remaining in the stomach were assayed. The content was a very small amount. Further, we observed the inner walls of stomach in full detail. The definite differences in the inner walls of stomach between the bovine bile group and the control were not recognized according to our observations. Similar results, as shown in Fig. 1B, were also observed by the concurrent intubation of ethanol and bovine bile. Bovine bile (0.4 g·kg⁻¹) decreased the $C_{\text{max}}$ to 0.63 ($p < 0.001$) and delayed the $T_{\text{max}}$ to 1.9 times ($p < 0.001$) compared with the control, whereas $T_{\text{disap}}$ showed no significant changes with the dose of bovine bile. These data were analyzed by Student’s $t$-test. Figure 1C shows the time courses of the plasma ethanol concentration when bovine bile (0.4 g·kg⁻¹) 30 min after oral intubation of ethanol (1.0 g·kg⁻¹) was administered orally. Both $C_{\text{max}}$ and $T_{\text{max}}$ did not change with the dose of bovine bile (0.4 g·kg⁻¹). $T_{\text{disap}}$ also did not change. Figure 1D shows the time courses of plasma ethanol concentration when ethanol (1.0 g·kg⁻¹) was intraperitoneally administered 30 min after oral intubation of bovine bile (0.4 g·kg⁻¹). In this condition, $C_{\text{max}}$, $T_{\text{max}}$ and $T_{\text{disap}}$ were independent of bovine bile.

Effects of dose of the bovine bile on the plasma ethanol levels

Figure 2 shows the time course of the plasma ethanol concentrations when 0, 50, 100, 200 or 400 mg·kg⁻¹ of the bovine bile 30 min before oral intubation of ethanol (1.0 g·kg⁻¹) were administered orally. The dose-dependent decrease in $C_{\text{max}}$ and delay of $T_{\text{max}}$ were observed by oral administrations of ethanol and bovine bile. $T_{\text{disap}}$ was insensitive to the change of the bovine bile dosage. Figure 3 shows the effects of various dosage of bovine bile on $C_{\text{max}}$ (A) and $T_{\text{max}}$ (B) as plotted

![Fig. 2. Time courses of plasma ethanol concentration for various dosages of bovine bile. Ethanol (1.0 g·kg⁻¹) was administered orally to the rats (8 weeks old) 30 min after gastric intubation of bovine bile. Each plasma sample was prepared from tail blood. The dose (mg·kg⁻¹) of the bovine bile=0 (n=9), 50 (n=7), 100 (n=5), 200 (n=7), and 400 (n=8) for ○, ●, △, ▲, and □, respectively. All data are shown as mean±SE.](image-url)

Fig. 3. Dose-response of bovine bile to each parameter. A: \(C_{\text{max}}\). B: \(T_{\text{max}}\). The detailed explanations of these parameters are described in METHODS. Each parameter is shown as mean±SE. C: The relation between \(C_{\text{max}}\) value and \(T_{\text{max}}\) value in the presence of various dosage of the bovine bile. All the data were employed from Fig. 2. The bovine bile dose (mg·kg\(^{-1}\)) = 0, 50, 100, 200, and 400 for ○, ●, △, ▲, and □, respectively. Data are shown as mean±SE.

against the bovine bile dose. The bovine bile of 50, 100, 200 and 400 mg·kg\(^{-1}\) decreased the \(C_{\text{max}}\) to 0.91, 0.81 (p<0.05), 0.78 (p<0.01) and 0.63 (p<0.01) times, respectively, compared with control value. These data were analyzed Dunnnett’s test. The \(T_{\text{max}}\) increased proportionally to the dose of bovine bile up to 200 mg·kg\(^{-1}\). The bovine bile of 50, 100, 200 and 400 mg·kg\(^{-1}\) delayed the \(T_{\text{max}}\) to 1.39, 1.44 (p<0.05), 1.50 (p<0.01) and 1.87 (p<0.01) times compared with that estimated in bovine bile-free rats, respectively. These data also were evaluated by Dunnnett’s test. Although not shown, the \(T_{\text{disap}}\) was independent of various bovine bile dose. Although not shown in the figure, \(k_{\text{(app)}}\) were constant (1.4–1.5×10\(^{-2}\) min\(^{-1}\)) in the dose range up to 400 mg·kg\(^{-1}\) of bovine bile. As shown in Fig. 3C, there was a negative correlation between \(C_{\text{max}}\) and \(T_{\text{max}}\).

Although not shown in the figures, we studied the effect of bovine bile with high osmotic pressure (above 450 mOsm·kg\(^{-1}\) H\(_2\)O) on plasma ethanol levels. Therefore, we analyzed the time course of the plasma ethanol concentration when 8% bovine bile with 450 or 550 mOsm·kg\(^{-1}\) H\(_2\)O 30 min before oral intubation of ethanol was administered orally. In these conditions, a marked decrease of plasma ethanol concentration was observed. The magnitude of the decrease was higher significantly than that observed in 8% bovine bile with 305 mOsm·kg\(^{-1}\) H\(_2\)O. Further, we also observed the internal organs such as stomach, duodenum and small intestine. Marked expansions and hemorrhage of gastrointestinal tracts (especially in stomach) were observed. From these results, it is concluded that the artifact of plasma ethanol level because of the abnormalities in the gastrointestinal tracts may be induced by the administration of bovine bile solution with a high osmotic pressure. Therefore, in the present study, the osmotic pressure of bovine bile solution was maintained to about 310–315 mOsm·kg\(^{-1}\) H\(_2\)O in order to exclude these artifacts.

As seen in Table 1, about 18.5% of bovine bile used was analyzed as total bile
Table 1. Bile acid composition of bovine bile used (mg·g⁻¹).

<table>
<thead>
<tr>
<th>Bile acid</th>
<th>Free-form</th>
<th>Conjugated-form</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glycine</td>
<td>Taurine</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>5.0</td>
<td>61.4</td>
<td>86.0</td>
</tr>
<tr>
<td>Chenodeoxycholic acid</td>
<td>0.2</td>
<td>7.0</td>
<td>11.4</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>0.4</td>
<td>11.9</td>
<td>0</td>
</tr>
<tr>
<td>Lithocholic acid</td>
<td>0</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Ursodeoxycholic acid</td>
<td>0</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Total</td>
<td>5.6</td>
<td>81.2</td>
<td>98.6</td>
</tr>
</tbody>
</table>

Each value was corrected by water content of the bovine bile.

acids. The bile acid composition of the bovine bile was in the following order: cholic acid (82.2%) > chenodeoxycholic acid (10.0%) > deoxycholic acid (6.6%) > lithocholic acid (0.7%) > ursodeoxycholic acid (0.5%). About 82% of total bile acids were cholic acid derivatives. The percentage of free-, glycine-conjugated and taurine-conjugated forms of total cholic acid was 2.7, 33.1 and 46.4%, respectively. Taurocholic acid was the major component of bile acid conjugates in the bovine bile (Table 1). Therefore, we examined whether taurocholic acid affects the plasma ethanol levels. The results are shown in Fig. 4. Taurocholic acid of 50 and 200 mg·kg⁻¹ decreased the Cₘₐₓ to 0.81 (p < 0.01) and 0.48 (p < 0.01) times, respectively, compared with the value of the control. These data were evaluated by Dunnett's test. On the other hand, 50 and 200 mg·kg⁻¹ of taurocholic acid delayed Tₘₐₓ to
1.35 and 2.08 times compared with the control, respectively. The relations between $C_{\text{max}}$ (A), $T_{\text{max}}$ (B) or $T_{\text{disap}}$ and dose of taurocholic acid, as shown in the insets of Fig. 4, showed dose-dependence. The $T_{\text{disap}}$ was independent of the dosage of taurocholic acid. These results were consistent with those obtained in the bovine bile, as shown in Figs. 2 and 3.

**Effects of bovine bile on ADH and ALDH (high $K_m$ and low $K_m$) activities**

There was no significant change in the ADH activities when the bovine bile group was statistically compared with the control (data not shown). When the mitochondrial high and low $K_m$ ALDH activities were compared with the control versus the bovine bile group, no significant differences in the both enzyme activities were observed (data not shown).

**Effect of bovine bile on gastric emptying**

As shown in Figs. 1A and B and Fig. 2, more effective time of the bovine bile to plasma ethanol level was 25–35 min after the intubation of ethanol. Therefore, the gastric emptying of ethanol was analyzed quantitatively at 10 and 30 min after intubation of ethanol. When bovine bile (0.4 g·kg$^{-1}$) 30 min before oral intubation of ethanol (1.0 g·kg$^{-1}$) was orally administered, the effects of bovine bile on the time course of the gastric emptying were as shown in Fig. 5. Figure 5A shows the

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**Fig. 5.** Effects of bovine bile on the time course of plasma ethanol concentration (A), content in stomach (B), ethanol concentration in stomach (C) and remaining rate of ethanol in stomach (D). Ethanol (1.0 g·kg$^{-1}$) was administered orally to the rats (6 weeks old) 30 min after oral intubation of bovine bile (0.4 g·kg$^{-1}$). The dose of bovine bile free ($n=8$ at each point) and 0.4 g·kg$^{-1}$ ($n=8$ at each point) for ○ and ●, respectively. Each value is represented as mean ± SE.
time courses of plasma ethanol concentration obtained in this condition. Clearly, a
significant decrease of plasma ethanol concentration was observed in the bovine bile
group. This result approximately agreed with those of Figs. 1A and 2. As shown
in Fig. 5B, the contents in stomach of the bovine bile group were 2.7 times (p <
0.001) higher than those of the control. Ethanol concentrations in stomach 10 and
30 min after intubation of ethanol were 1.6 times (p < 0.05) and 3.4 times (p <
0.001) higher, respectively, in the bovine bile group than in the control. The degree
of the decrease of ethanol concentration in stomach from 10 to 30 min after
intubation of ethanol was 1.7 times higher in the control than in the bovine bile
group (Fig. 5C). The remaining rates of ethanol in stomach are shown in Fig. 5D.
The remaining rates of ethanol in stomach of the bovine bile group were 5.6 times
(p < 0.001) and 8.7 times (p < 0.001) higher for 10 and 30 min, respectively, than
the control. These data were analyzed by Student's t-test.

**Effect of bovine bile on the intestinal absorption rate of ethanol**

The intestinal absorption rates of ethanol from *in situ* ligated loop was 2.06 ±
0.18 and 1.42 ± 0.21 mg·min⁻¹·cm⁻¹ loop for the control and the bovine bile group,
respectively. The bovine bile decreased the intestinal absorption rate of ethanol to
0.69 times (p < 0.001) compared with the control.

**DISCUSSION**

The present paper clearly showed that bovine bile changed plasma ethanol
levels (Figs. 1–3). Further, the present study suggests that the intestinal absorption
may be involved in the effects of bovine bile on plasma ethanol level *in vivo*. No
changes in the hepatic ADH and ALDH activities were observed by bovine bile,
suggesting that bovine bile alters absorption of ethanol rather than its metabolism
after absorption. In fact, the contents and concentrations of ethanol remaining in
stomach 10 and 30 min after ethanol intubation were much higher with adminis-
tration of the bovine bile (Fig. 5). Further, as shown in Fig. 6, there was a negative
correlation between plasma ethanol concentration and gastric content (A), gastric
ethanol concentration (B) or the remaining rate of ethanol in stomach (C). These
results suggest that the bovine bile strongly slows gastric emptying of ethanol in
rats.

Generally, ethanol can be absorbed into the body through the whole of the
gastrointestinal tract from the mouth to the rectum. The main regions for
absorption are the duodenum and jejunum, with a lower but appreciable uptake
from the stomach and the large intestine and minimal absorption from the mouth
(17). There is a general consensus of opinion that ethanol is transferred across
biological membranes by a process of simple diffusion (17). There do not seem to
be any permeability barriers to the movement of ethanol through all the water
compartments of the body, nor any active uptake mechanisms.

On the other hand, the amount of ethanol absorbed through the gastric mucosa

will be dependent, in part, on the level of retention of the alcoholic liquor in the stomach (17). If the pylorus allows the rapid movement of the alcohol through to the jejunum and duodenum, absorption through the stomach walls would be expected to represent only a minor route for ethanol to enter the body water compartments (17).

The present paper also showed that the small intestinal absorption rate of ethanol was decreased significantly by bovine bile. From these results, it is suggested that the delay of the gastric emptying and/or the decrease of the absorption of ethanol in the gastrointestinal tract are a major mechanism for the plasma ethanol decreasing and delaying effects of bovine bile. At the present moment, a precise mechanism of this phenomenon is not elucidated. The intestinal loop used in the present study was the proximal portion of the small intestine. Generally, ethanol is absorbed in the proximal small intestine under normal physiological circumstances (18). In this loop, the active transport system of bile acids in the sample through mucosal membrane may be neglected because the active transport system of bile acids is located in the distal ileum (19). Moreover, taurocholic acid, which is the major component of bile acid in bovine bile, produced qualitatively the same actions as bovine bile (Table 1 and Fig. 4).

Generally, bile acid salts in animal bile have properties as ionic detergents (20–22). It is also reported that ionic detergents such as bile acids, fatty acids, and chelators stimulated the membrane of the duodenal receptors and slowed gastric emptying through the binding of ionized calcium (22). Furthermore, it is proposed that several ionic detergents slowed gastric emptying by decreasing the dimensions of the lateral intercellular space between the duodenal enterocytes (22). This space gives a signal that indirectly slows gastric emptying through nerves or hormones such as gastrin, cholecystokinin, secretin, gastric inhibitory peptide (GIP), glucagon, and vasoactive intestinal polypeptide (VIP) (22–25). It is well known that
various dietary components such as carbohydrates, proteins, fats, amino acid, and acids, delay gastric emptying (22,26–29). For instance, the slowing of gastric emptying by fats in food is considered to be mediated by the anions of long-chain fatty acids set free during the digestion of the triglycerides in the duodenum (22).

As already described, taurocholic acid changed plasma ethanol levels. The $pK$ of taurocholic acid is about 1.4. The sulfonic group of taurocholic acid exists as an anion to ionize in the stomach (30). Therefore, we suppose that the slowing of the gastric emptying by bovine bile is in part mediated by the interaction between the anion of the sulfonic group of taurocholic acid and duodenal receptors. Since the rate of transfer of ethanol from the stomach to the small intestine markedly influences the rate at which ethanol is absorbed, the motility of the stomach is often a major rate-limiting factor in ethanol absorption (18,31–33). In this case, the rate of absorption of ethanol from the small intestine depends on the rate of gastric emptying (18). Therefore, when gastric emptying is slow, the absorption of ethanol is delayed and peak plasma ethanol concentration is reduced (33). Alterations of the gastric emptying rate, which may have a pharmacologic, physiologic or pathologic cause, markedly influence the rate of ethanol absorption (33,34). The gastric emptying rate makes an important contribution to inter- and intra-individual variations in the rate of ethanol absorption and therefore the timing and magnitude of the acute intoxication effect of an oral dose of ethanol (33,35). According to our unpublished results, the contents of total fatty acid and total cholesterol of bovine bile were estimated as 37.0 and 0.5%, respectively. The contribution and magnitude of each substance to the changing actions of plasma ethanol level by bovine bile are not clear. These possibilities need further elaborate studies.

The excretion of ethanol from the urine or expiration must be considered as other factors which affect the plasma ethanol concentration. These factors could not be determined directly in this study. However, it is indicated that the proportion of ethanol excreted or expired directly was smaller, and that there was little significance (36). Therefore, these factors seem less important.

In conclusion, it is suggested that the plasma ethanol levels decreased by bovine bile correlate significantly with the delay of gastric emptying and/or the decrease of intestinal absorption rate of ethanol. The present paper also suggests that bile acid such as taurocholic acid may participate in the changing actions of plasma ethanol level by bovine bile.

REFERENCES


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