Effects of Tea (Camellia sinensis) Chemical Compounds on Ethanol Metabolism in ICR Mice

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The effects of the green tea (Camellia sinensis) extract on ethanol metabolism in ICR male mice were studied. A crude green tea extract (GTE) and the tea components as (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), and caffeine were administered before the tests. One hour later, the mice were orally given 2 g/kg body weight (b.w.) of ethanol (20% ethanol w/v). The results show that the levels in the blood and liver of ethanol and acetaldehyde were lower, and that the levels of acetate and acetic acid were higher than in the controls orally given 500 mg/kg b.w. of GTE. After the administration of 75 mg/kg b.w. and 225 mg/kg b.w. of EGCG, the acetate and acetic acid concentrations in the blood and liver were lower than in the controls. The mice given caffeine at the same dose as that in GTE showed almost the same effects as the group treated with GTE. This suggests that EGCG and caffeine, the principal components of GTE, both had an effect on ethanol metabolism.

Key words: ethanol metabolism; tea; (−)-epigallocatechin gallate; caffeine

An excessive intake of alcohol causes hangover symptoms like headache and discomfort, even though drinking in moderation may not be harmful. This symptom is thought to be due to the combined effects of acetaldehyde, acetate, and ketone bodies which are all ethanol metabolites. The reported studies have dealt with the detoxication effects of herbal medicine, amino acids, fructose, and food stuffs in cases of acute alcoholism.

The suggestion of an improvement to the discomfort from excessive alcohol ingestion by drinking tea has already been recorded by Eisai-Zenshi in the Kamakura era in Japan. Thus, the effects of tea on acute alcoholism have been studied in Japan from early times. Tea leaves contain many substances like catechins, theanine, and some other reportedly biologically revitalizing chemicals.

Yet, there has been little research on the effects of tea components on alcohol metabolism, except for the inhibition of ethanol absorption by tea seed saponine. We studied the influence of a crude green tea (Camellia sinensis) extract and its main components, catechins and caffeine, on alcohol metabolism. We used for these studies ICR mice with a high resistance to alcohol and orally administered tea extracts 1 h before administering ethanol. Sequential changes in the blood and liver concentration of ethanol, acetaldehyde, acetate, and acetic acid were simultaneously measured by headspace-gas chromatography.

Materials and Methods

Preparation of the test material. Green tea was extracted from 1 kg of intermediate-grade green tea leaves with 20 liter of hot water for 30 min. The extract was fractionated by using Diaion HP-20 resin with styrene divinylbenzene (Mitsubishi Kagaku, Tokyo, Japan). A glass column of 100 dia. x 1000 mm length was packed with 2.5 liters of HP-20 resin and washed with methanol and distilled water before use. A concentrated green tea extract (5 liters) was passed through the column, and the unadsorbed fraction was washed out with a six-fold volume of distilled water. The fractions adsorbed to the HP-20 resin were eluted with a five-fold volume of methanol. Next, the fractions were concentrated, freeze-dried and powdered (GTE). The percentage yield of GTE was 7.0, and Table shows the components of GTE.

Table. Components of the Crude Green Tea Extract

<table>
<thead>
<tr>
<th>Component</th>
<th>Content (% w/w of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechins</td>
<td></td>
</tr>
<tr>
<td>(−)-Epigallocatechin</td>
<td>16.61</td>
</tr>
<tr>
<td>(−)-Epigallocatechin gallate</td>
<td>13.72</td>
</tr>
<tr>
<td>(−)-Epicatechin</td>
<td>3.40</td>
</tr>
<tr>
<td>(−)-Epicatechin gallate</td>
<td>2.19</td>
</tr>
<tr>
<td>Caffeine</td>
<td>9.93</td>
</tr>
<tr>
<td>Amino acids</td>
<td></td>
</tr>
<tr>
<td>t-aspartic acid</td>
<td>0.26</td>
</tr>
<tr>
<td>t-glutamic acid</td>
<td>0.30</td>
</tr>
<tr>
<td>t-asparagine</td>
<td>0.05</td>
</tr>
<tr>
<td>t-serine</td>
<td>0.08</td>
</tr>
<tr>
<td>t-glutamine</td>
<td>0.11</td>
</tr>
<tr>
<td>t-arginine</td>
<td>0.13</td>
</tr>
<tr>
<td>t-alanine</td>
<td>0.04</td>
</tr>
<tr>
<td>t-methionine</td>
<td>0.04</td>
</tr>
<tr>
<td>t-theanine</td>
<td>0.97</td>
</tr>
<tr>
<td>γ-amino butyric acid</td>
<td>0.05</td>
</tr>
<tr>
<td>Others</td>
<td>52.14</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
</tr>
</tbody>
</table>

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individually in stainless steel mesh cages in an air-conditioned room (20 ± 2 °C, 60 - 65% humidity) with a 12-h light and dark cycle (lighting from 6:00 to 18:00). The mice had free access to water and to a basal diet (CE-2, Clea Japan, Tokyo, Japan) for one week to accustom them to the surroundings.

Sample and ethanol schedules. The mice were divided into two groups for the control and test materials, six to ten mice being assigned to each group. The animals were fasted on the day before the experiment. Ethanol at a concentration of 20% (v/v) was given orally through a gastric catheter at a dose of 2 g/kg b.w. The control group received only ethanol. The experimental group was prepared by administering 500 mg/kg b.w. of GTE, 75 or 225 mg/kg b.w. of EGCg, 170 mg/kg b.w. of EGC, and either 50 or 300 mg/kg b.w. of caffeine in the form of 1% (w/v) solutions in distilled water prior to administering the ethanol. The control group received distilled water instead of the tea extract.

Analytical procedure. One hour later, the animals were orally fed with 2 g/kg b.w. of ethanol (20% ethanol w/v). Blood and liver samples were then obtained 1 and 3 h after the ethanol administration. The ethanol, acetaldehyde, acetate, and acetone concentrations in these specimens were measured according to the procedures devised by Tsukamoto et al. [20.21]. About 0.5 ml of blood was immediately mixed with 2.5 ml of an ice-chilled PCA reagent (0.6% perchloric acid, 30 mM thiourea, and 0.1 mM EDTA in saline). The 0.5 g liver specimen was immediately powdered in liquid nitrogen for subsequent deproteinization by the PCA reagent. The samples were then centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was poured into vials as follows: 1 ml into vial 1 for the measurement of ethanol, acetaldehyde, and acetone; and another 1 ml for the measurement of acetate together with 80 μl of methanol and 50 μl of sulfuric acid were poured into vial 2 to methylate the acetate. An amount of 0.1 ml of 0.0003% n-butanol was put into vial 1 and 0.1 ml of 0.02% n-butanol into vial 2 to serve as internal standards. After heating vial 1 at 65 °C for 15 min and vial 2 at 55 °C for 30 min, the headspace gas of each was introduced into a gas chromatograph (Perkin-Elmer, HSGC-101). The following conditions of Tsukamoto et al. [20.21] for gas chromatography were used to measure ethanol, acetaldehyde, acetate, and acetone: column, 4.0 mm dia. x 4 m glass, 10% PEG 600 Chromosorb W, 80-100 mesh; temperatures, 68°C for the column (75°C for methylacetate), 140°C for the injector; N₂ pressure, 30 psi; H₂ pressure, 18 psi; air pressure, 18 psi; range, 1.

Statistical analysis. All data were analyzed by a one-way analysis of variance. A subsequent examination of the statistical significance of the difference of the means was evaluated by Student’s t test between two groups at a level of significance of p<0.01.

Results

Changes in ethanol, acetaldehyde, acetate, and acetone concentrations in the blood

Figure 1 shows the concentration changes in ethanol, acetaldehyde, acetate, and acetone in the blood after the oral administration of 2 g/kg b.w. of ethanol (20% ethanol w/v). One hour before the ethanol administration, 500 mg/kg b.w. of GTE was given orally. After 3 h, the ethanol and acetaldehyde concentrations were lower than those in the control group (Figs. 1A and 1B). Compared with the control group both the acetate (Fig. 1C) and acetone (Fig. 1D) concentrations increased markedly with time.

Fig. 1. Changes in the Concentrations of Ethanol, Acetaldehyde, Acetate, and Acetone in the Plasma after an Oral Administration of the Crude Green Tea Extract (GTE).

Fig. 2. Changes in the Concentrations of Acetate and Acetone in the Plasma after an Oral Administration of Catechins.

- control
- GTE: The mice were given 500 mg of GTE/kg body weight by gastric intubation 1 h before the ethanol administration (2 ml of 20% ethanol kg b.w.). Values represent means ± SEM (n=6).
- * shows a significant difference as compared to the corresponding control at p<0.01.

- control
- EGC (170 mg/kg b.w.); EGCg (75 mg/kg b.w.); EGC (225 mg/kg b.w.); EGCg (225 mg/kg b.w.). The mice were given 170 mg of EGC kg b.w., 75 mg of EGCg kg b.w., or 225 mg of EGCg kg b.w. by gastric intubation 1 h before the ethanol administration (2 ml of 20% ethanol kg b.w.). Values represent means ± SEM (n=8).
- * shows a significant difference as compared to the corresponding control at p<0.01.

Figure 2 shows the concentration changes in ethanol, acetaldehyde, acetate, and acetone in the blood after the oral administration of 2 g kg\(^{-1}\) b.w. of ethanol (20% ethanol w/v). One hour before the ethanol administration, 170 mg kg\(^{-1}\) b.w. of EGCG and either 75 or 225 mg kg\(^{-1}\) b.w. of EGCG were given orally. The variations in ethanol and acetaldehyde concentrations did not differ from those in the control (Figs. 2A and 2B). The acetate level in the group given EGCG was lower than that in the control group at 3 h (Fig. 2C). The acetone level in the group given 225 mg kg\(^{-1}\) b.w. of EGCG was significantly lower than that in the control group (Fig. 2D).

Figure 3 shows the concentration changes in ethanol, acetaldehyde, acetate, and acetone in the blood after the oral administration of 2 g kg\(^{-1}\) b.w. of ethanol (20% ethanol w/v). One hour before the ethanol administration, 50 or 300 mg kg\(^{-1}\) b.w. of caffeine was given orally. The ethanol concentration in the group given 300 mg kg\(^{-1}\) b.w. of caffeine was significantly lower after 1 and 3 h (Fig. 3A) than that in the control group. Caffeine administration did not alter the acetaldehyde concentration (Fig. 3B). In the group treated with 300 mg kg\(^{-1}\) b.w. of caffeine, the acetate and acetone concentrations increased markedly after 1 and 3 h (Figs. 3C and 3D).

Changes in the ethanol, acetaldehyde, acetate, and acetone concentrations in the liver

Figure 4 shows the changes in ethanol, acetaldehyde, acetate, and acetone concentrations in the liver after the oral administration of 2 g kg\(^{-1}\) b.w. of ethanol (20% ethanol w/v). One hour before the ethanol administration, 500 mg kg\(^{-1}\) b.w. of GTE was given orally. After 3 h, the ethanol level was lower than that in the control group (Fig. 4A). After 1 h and 3 h, the acetaldehyde level was also lower than that in the control group (Fig. 4B). Compared with the control group acetate (Fig. 4C) was significantly increased after 1 h and 3 h, while the acetone concentration (Fig. 4D) increased markedly over the time.

Figure 5 shows the changes in ethanol, acetaldehyde, acetate, and acetone concentrations in the liver after the oral administration of 2 g kg\(^{-1}\) b.w. of ethanol (20% ethanol w/v). One hour before the ethanol administration, 170 mg kg\(^{-1}\) b.w. of EGCG and either 75 or 225 mg kg\(^{-1}\) b.w. of EGCG were given orally. The variations in ethanol and acetaldehyde concentration did not differ from those in the control group (Figs. 5A and 5B). After 3 h, the acetate and acetone concentrations in the group given EGCG were lower than those in the control group (Figs. 5C and 5D).
Figure 6 shows the changes in ethanol, acetaldehyde, acetate, and acetone concentrations in the liver after oral administration of 2 g/kg b.w. of ethanol (20% ethanol w/v). One hour before the ethanol administration, 50 or 300 mg/kg b.w. of caffeine was given orally. After 1 h and 3 h (Figs. 6A and 6B), the ethanol and acetaldehyde concentrations were markedly lower than those in the control group. The acetate concentration after 3 h was higher than that in the control group (Fig. 6C), while it was markedly increased from the beginning to 3 h later compared with control group (Fig. 6D). The effect of 300 mg/kg b.w. of caffeine was stronger than that of 50 mg/kg b.w.

**Discussion**

In this study, 2 g/kg b.w. of ethanol (20% ethanol w/v) was given orally to ICR male mice that had previously received 1 h earlier at 500 mg/kg b.w. of the green tea extract (GTE) that had been extracted from intermediate-grade green tea leaves. In this case, the concentrations of ethanol and acetaldehyde in the blood and liver were lower than those in the control group, but the concentrations of acetate and acetone were higher than those in the control group.

These results suggest that GTE promoted alcohol metabolism. The GTE used in this experiment contained high concentrations of catechins\(^1\) \(^2\) \(^3\) \(^4\) and caffeine\(^2\) \(^0\) \(^1\) \(^2\) \(^3\), which are known to have various biological activities. As though no reports have dealt with the influence of tea components on alcohol metabolism, the influence of catechins and caffeine on this metabolism have been suggested.

When caffeine was given to ICR mice, the acetate and acetone concentrations in the blood and liver were markedly higher than those in the control group. The significantly increased acetate and acetone concentrations probably caused the acidic plasma. On the other hand, when EGCG in catechins was given orally to the ICR mice, the acetate and acetone levels in the blood and liver tended to decrease compared with those in the control group. This observation suggests that EGCG had an antioxidative effect on acid plasma after excessive ethanol ingestion. Since the antioxidative effect of catechins\(^1\) \(^2\) and their absorption\(^2\) \(^3\) through the intestine are known, these results also suggest a detoxification effect.

The GTE used in this study also contained about 10% of caffeine. The results show that the ethanol concentration in the blood and liver of the group given 300 mg/kg b.w. of caffeine was significantly lower than that in the control group. This suggests that caffeine extracted a promotive effect on alcohol metabolism.

More than 95% of acetaldehyde during ethanol metabolism is metabolized in the liver and reportedly is distributed in the order of kidney > liver > blood > brain.\(^2\)\(^3\)
In the present study, similar results were obtained when the acetaldehyde level in the liver was higher than that in the blood. Even after a dose of 50 mg/kg b.w. of caffeine, the acetaldehyde concentration in the liver after 1 h was markedly lower than that in the ethanol control. This suggests that the prevention of an increased acetaldehyde concentration in the liver after being orally given GTE depends on the influence of the caffeine content in GTE.

The acetaldehyde metabolite, acetate, is usually degraded in the TCA cycle to carbon dioxide and water through CoASAc. This suggests that the administration of 2 ml/kg b.w. of 20% (w/v) ethanol used in this study represented an overdose of ethanol that was a burden upon the liver. This shows that the alcohol metabolism interfered with the metabolism of acetate to CoASAc, leading to an increased acetate level.

Moreover, above the level in the control group, the acetate levels in the blood and liver were intentionally increased by administering caffeine. Thus, the administration of caffeine apparently affected the acetaldehyde in the liver, promoted oxidation, and consequently raised the acetate concentration above that in the control group. Accordingly, the increased concentrations of acetate in the blood and liver suggest the influence of the administered green tea extract.

The mice receiving caffeine had significantly higher acetone levels in the liver than in the control group. This observation is similar to that resulting from an increase of cAMP concentration in fat cells, hormone-sensitive lipase then being activated and the decomposition of fat stored being promoted; moreover, caffeine promoted the hydrolysis of fat and increased the plasma concentration of fatty acids. Therefore, administration of caffeine apparently affected fat stored in the fat cells and increased the acetone concentration level. These results suggest that green tea components affected alcohol and lipid metabolism.

GTE contained about 1% of theanine, so we gave the mice in this study a dose of 50 mg/kg b.w. of theanine, about 10% being orally. However, the effect of theanine on alcohol metabolism remains unclear. Theanine has a caffeine antagonistic effect and may be used in combination with caffeine to control excitation effects of caffeine on the central nervous system.

References