Green Tea Suppresses Lipopolysaccharide-Induced Liver Injury in \( \text{d-Galactosamine} \)-Sensitized Rats\(^1\)

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ABSTRACT We conducted a series of in vivo experiments to clarify the hepatoprotective activity of green tea against lipopolysaccharide (LPS) + \( \text{d-galactosamine} \) (GalN)-induced liver injury and to elucidate the mechanism by which green tea exerts its effect in 7-wk-old male Wistar rats. Liver injury was assessed by plasma alanine aminotransferase and aspartate aminotransferase activities. Green tea extract significantly suppressed LPS + GalN-induced liver injury when added to the diet (30 or 35 g/kg) and fed to rats for 14 d or when force-fed alone (0.4–1.2 g/kg body) 1.5 h before the injection of drugs. Although all five of the fractions extracted from green tea extract with different organic solvents had significant suppressive effects, the caffeine-containing fraction exhibited the strongest effect, suggesting that the protective effect of green tea against LPS + GalN-induced liver injury is attributable mainly to caffeine. Authentic caffeine also significantly suppressed LPS + GalN-induced liver injury when added to the diet (2 g/kg) and fed to rats for 14 d. Dietary green tea suppressed LPS + GalN-induced apoptosis of liver cells, as assessed by DNA fragmentation. However, dietary green tea did not suppress LPS-induced enhancement of plasma concentration of tumor necrosis factor (TNF)-\( \alpha \), the cytokine that is thought to play a pivotal role in the pathogenesis of LPS-induced liver injury, although it significantly suppressed plasma concentrations of interleukin (IL)-1\( \beta \), IL-2, IL-4, IL-6, IL-10 and interferon (IFN)-\( \gamma \). TNF-\( \alpha \) + GalN-induced liver injury and apoptosis were also suppressed by dietary green tea. In contrast, dietary caffeine significantly suppressed LPS-induced enhancement not only of plasma IL-1\( \beta \), IL-6, IL-10 and IFN-\( \gamma \) concentrations, but also of TNF-\( \alpha \) concentration. The results suggest that green tea might suppress LPS + GalN-induced liver injury mainly through the inhibition of TNF-\( \alpha \)-induced apoptosis of hepatocytes, rather than through the suppression of TNF-\( \alpha \) production, although the suppressed production of TNF-\( \alpha \) may be associated with the hepatoprotective effect of caffeine. J. Nutr. 131: 1560–1567, 2001.

KEY WORDS: • green tea • liver injury • caffeine • lipopolysaccharide • apoptosis • rats

Traditional beverages are not necessarily major sources of nutrients, except for green tea, which contains relatively large amounts of vitamin C. However, a number of studies have shown that traditional beverages, as represented by teas, are beneficial to human health (Trevisanato and Kim 2000), suggesting that these beverages might warrant investigation from the viewpoint of nutrition. To date, a number of studies have shown that green tea or its constituents possess a variety of effects, including antioxidation (Matsuzaki and Hara 1985), antimutation (Kada et al. 1985), anticarcinogenesis (Fujiki et al. 1996), antibiotic action (Toda et al. 1989), antihypercholesterolemia (Muramatsu et al. 1986), antihypertension (Hara and Tonooka 1990), antihyperglycemia (Shimizu et al. 1988) and anti-inflammatory action (Sagesaka et al. 1996). In addition, we demonstrated that green tea could suppress \( \text{d-galactosamine} \) (GalN)\(^3\)-induced liver injury in rats (Sugiyama et al. 1998). Our recent studies showed that the protective effect of green tea against GalN-induced liver injury was ascribed mainly to flavonoid glycosides, theanine and soluble dietary fibers, whereas the effect of tea catechins (tea polyphenols), characteristic constituents of teas, was relatively weak (He et al. 2000, Sugiyama et al. 1999, Wada et al. 1999). In contrast, Yang et al. (1998) reported that tea catechins may inhibit lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)-\( \alpha \) production in both mouse peritoneal macrophages and mice in vivo. LPS is a component of the outer membrane of gram-negative bacteria, which have been used frequently in combination with GalN to induce liver injury in rodents. These results suggest that tea catechins may be effective in suppressing liver injury induced by LPS because LPS is known to cause liver injury through enhanced TNF-\( \alpha \) production (Tiegs et al. 1989). However, it is unclear whether tea or its constituents, including tea catechins, actually suppress LPS-induced liver injury. On the other hand, synthetic methylxanthine derivative drugs, e.g., pentoxifylline [3,7-dimethyl-1-(5-oxohexyl)xanthine] and A802715 [1-(5-hydroxy-5-methyl)hexyl-3-methyl-7-propylxanthine], have been shown to inhibit TNF-\( \alpha \) production, thereby suppressing LPS-induced liver injury (Fischer et al. 1993, Jilg et al. 1996, Sinha et al. 1995). These findings suggest the possibility that caffeine, the most common naturally occurring methylxanthine,
may also have a suppressive effect on LPS-induced liver injury. However, the hepatoprotective effect of caffeine against LPS-induced liver injury has not been fully demonstrated.

In this study, we first examined whether green tea extract could protect rats from LPS-induced liver injury, as assessed by plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in GaIN-sensitized rats. Because green tea had a suppressive effect, the effects of five fractions, which were prepared from the green tea extract by successive extraction with different organic solvents, were investigated to determine what type of constituent contributes to the effect of green tea. Furthermore, we investigated the effects of dietary green tea extract on LPS-enhanced plasma concentrations of various kinds of cytokines, including TNF-α, and on the apoptosis of liver cells in GaIN-sensitized rats to gain insight into the mechanism by which green tea suppresses LPS-induced liver injury. The effects of green tea extract on TNF-α-induced liver injury and of caffeine on plasma cytokine concentrations were also investigated.

MATERIALS AND METHODS

Materials. The green tea (Sen-cha) we used was obtained from a market (Shizuoka, Japan). Lipopolysaccharide from *Escherichia coli* 055 and GaIN hydrochloride were purchased from Wako Pure Chemical (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO), respectively. Rat recombinant TNF-α was purchased from Funakoshi (Tokyo, Japan). Mineral and vitamin mixtures (AIN-76) were purchased from Oriental Yeast (Tokyo, Japan).

Extraction and fractionation of green tea. Green tea was extracted as follows. 10 volumes (v/wt) of boiling water was added to tea, allowed to stand for 30 min at room temperature and the extract was filtered through five sheets of gauze. The extract was lyophilized and powdered with a mixer. The dry matter thus obtained was 23.5 g/100 g of green tea. The powder was further fractionated into five fractions (fractions I–V) by successive extraction with chloroform, ethyl acetate, n-butanol; EtOH, ethanol.

**FIGURE 1**

**TABLE**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr. I</td>
<td>CHCl₃ layer</td>
</tr>
<tr>
<td>Fr. II</td>
<td>Water layer</td>
</tr>
<tr>
<td></td>
<td>AcOEt layer</td>
</tr>
<tr>
<td>Fr. III</td>
<td>n-BuOH layer</td>
</tr>
<tr>
<td></td>
<td>Water layer</td>
</tr>
<tr>
<td></td>
<td>Added with 2.5 vol of ETOH</td>
</tr>
<tr>
<td>Fr. IV</td>
<td>Filtered</td>
</tr>
<tr>
<td>Fr. V</td>
<td>Residue</td>
</tr>
</tbody>
</table>

The procedure for the fractionation of green tea extract into five fractions (Fr. I–V) by successive extraction with different organic solvents. The yield of each fraction is shown in parentheses. AcOEt, ethyl acetate; n-BuOH, n-butanol; ETOH, ethanol.

AcOEt, ethyl acetate; EtOH, ethanol.

Animals and diet. Male Wistar rats, 5 wk old, weighing 90–120 g (Experiments 1, 2, 4–6) or 6-wk-old male Wistar rats weighing 120–140 g (Experiment 3) were obtained from Japan SL C (Hamamatsu, Japan). The rats were housed individually in hanging stainless steel wire cages and kept in an isolated room at a controlled temperature (23–25°C) and ambient humidity (50–60%). Light was maintained on a 12-h light:dark cycle (lights on 0600 to 1800 h). After rats were acclimated to the facility for 4 or 5 d and given free access to water and a commercial stock diet (Type MF; Oriental Yeast), they were fed the semipurified experimental diets. Body weight and food consumption were measured daily. The composition of the control diet was as follows (g/kg): casein, 250; cornstarch, 402.5; sucrose, 200; corn oil, 50; mineral mixture (AIN 1977), 35; vitamin mixture (AIN 1977), 10; choline bitartrate, 2.5; and cellulose, 50. Supplements were added to the control diet at the expense of cellulose.

In this study, six separate experiments were conducted. In Experiment 1, rats were fed either the control diet or a diet supplemented with powder of the green tea extract at 30 g/kg. In Experiment 2, rats were fed either the control diet or diets supplemented with one of the five fractions derived from the green tea extract. The addition levels of each fraction (Fr.) were as follows (g/kg diet): Fr. I, 2.15; Fr. II, 8.16; Fr. III, 4.64; Fr. IV, 10.71; and Fr. V, 4.34. In Experiment 3, rats were fed the control diet and force-fed water or a green tea extract alone by stomach tube at levels of 0.4, 0.8 and 1.2 g/kg body 1.5 h before the injection of drugs. In Experiment 4, rats were divided into two groups and fed the control diet or a green tea extract-supplemented (35 g/kg) diet. In Experiment 5, rats were divided into three groups and fed the control diet or a caffeine-supplemented (2 g/kg) diet. In Experiment 6, rats were divided into three groups and fed the control diet or a green tea extract-supplemented (35 g/kg) diet. Normal and control rats were fed the control diet.

After the experimental diets were fed for 10 (Experiment 3) or 14 d (Experiments 1, 2, 4 and 5), rats were injected intraperitoneally with LPS (10 μg/kg) and GaIN (250 mg/kg) on d 11 or 15. In Experiment 6, rats were injected intravenously with TNF-α (8 μg/kg) via the tail vein 20 min after an intraperitoneal injection of GaIN (250 mg/kg) on d 15. Untreated rats were injected with saline. In Experiments 1, 2 and 3, rats were killed by decapitation 22 h after the injection of drugs. In Experiment 4, rats were killed just before the injection (time 0) and at 1, 3, 6, 12 and 24 h after injection of LPS and GaIN. In Experiment 5, rats were killed 1 and 8 h after injection of drugs. In Experiment 6, rats were killed 9 h after injection of drugs.

Blood plasma was separated from heparinized whole blood by centrifugation at 2000 × g for 20 min at 4°C; the plasma and liver were stored at −80°C until analyses. The experimental design of this study was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture, Shizuoka University.

Biochemical analysis. The activities of plasma ALT and AST, the marker enzymes of liver injury, were measured with a kit (Transaminase C II-Test, Wako). The enzyme activity was expressed as μmol or mmol (min · L plasma) at 25°C. Plasma concentrations of interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-10, interferon (IFN)-γ, macrophage inflammatory protein (MIP)-2, and TNF-α were measured with Rat IL-1β ELISA Kit (Wako), Rat IL-2 ELISA Kit (Wako), Rat IL-4 ELISA Kit (Wako), Rat IL-6 ELISA Kit (Wako), Rat IL-10 ELISA Kit (Wako), Rat IFN-γ ELISA Kit (Wako), Rat MIP-2 ELISA Kit (Wako), and Rat TNF-α ELISA Kit (Cosmo Bio, Tokyo, Japan), respectively. Hepatic DNA fragmentation was detected and quantified by agarose gel electrophoresis and DNA ELISA, respectively, according to Leist et al. (1995) and Hase et al. (1999) with slight modifications. In brief, frozen liver (−1 g) was homogenized in 4 volumes (v/wt) of ice-cold PBS containing 10 mmol/L EDTA. The homogenate was centrifuged at 13,000 × g for 20 min at 4°C, and total DNA was prepared from the supernatant (0.3 mL) by extraction with an equal volume of phenol-chloroform (1:1, v/v), precipitation in ethanol and subsequent treatment with 20 mg/L of ribonuclease A (Sigma-Aldrich) for 30 min at 37°C. The total DNA was electrophoresed on 1.8% agarose gel. An aliquot of the supernatant of liver homogenate was diluted and subjected to the direct analysis of oligonucleosome-bound DNA with an ELISA kit (Boehringer Mannheim, Mannheim, Germany).

Statistical analysis. Results were expressed as means ± SEM. In Experiment 5, paired data at each time were analyzed by Student’s t test. In other experiments, data were tested for homogeneity of variance using Bartlett’s test and, if necessary, were log-transformed to homogeneity before one-way ANOVA. The difference between the means was tested at P < 0.05 using Duncan’s multiple range test.
(Duncan 1957) when the F-value was significant at \( P < 0.05 \). In Experiments 1, 2, 4 and 5, the effects of body weight or body weight gain of rats on ALT and AST activities were analyzed by an analysis of covariance.

**RESULTS**

**Effect of dietary green tea extract on LPS-induced liver injury (Experiment 1).** Green tea extract (30 g/kg) slightly depressed the body weight gain and food intake of rats compared with those fed the control diet (Table 1). Injection of LPS + GalN significantly increased plasma ALT and AST activities compared with saline-injected normal rats. The enhanced ALT and AST activities were significantly suppressed by dietary supplementation with the green tea extract. There was no correlation between body weight (or body weight gain) and the activities of ALT or AST in each dietary group, suggesting that the two variables are independent (data not shown). Similarly, no association was detected between the final body weight or body weight gain and the enzyme activities in the subsequent experiments (2, 4 and 5).

**Effect of each fraction from green tea extract (Experiment 2).** HPLC showed that the green tea extract used in this study contained 5.90 g caffeine and 28.2 g catechins/100 g, and that the main constituents of fractions I and II were caffeine and catechins, respectively; fraction I contained 88.5 g caffeine and a trace amount of catechins, whereas fraction II contained 1.0 g caffeine and 88.5 g catechins/100 g. Body weight gain was slightly depressed by dietary supplementation only with fraction I compared with rats fed the control diet; the values for normal rats, control rats and rats fed the fractions I, II, III, IV and V were 65 ± 2 (n = 6), 64 ± 1 (n = 12), 49 ± 2 (n = 10), 61 ± 3 (n = 10), 65 ± 3 (n = 10), 67 ± 2 (n = 10), 64 ± 2 g/14 d (n = 10), respectively. Food intake was also slightly depressed only by fraction I (data not shown). These data are essentially in agreement with our previous results (Sugiyama et al. 1999). Although all five of the fractions significantly suppressed the LPS + GalN-induced enhancement of plasma ALT and AST activities, the effect of fraction I was significantly stronger than those of the other fractions (Fig. 2); fraction I lowered the plasma enzyme activities to about one third of the value of control rats.

**FIGURE 2** Effects of dietary supplementation with each fraction (Fr.) obtained from green tea extract on lipopolysaccharide-induced enhancement of plasma alanine aminotransferase (A) and aspartate aminotransferase (B) activities in d-galactosamine-sensitized rats (Experiment 2). Normal rats were injected with saline. The column and its bar represent the mean value and SEM, respectively, for 6 (normal), 12 (control) or 10 (Fr. I–V) rats. Values with different letters are significantly different at \( P < 0.05 \). ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**Effect of force-feeding of green tea extract alone (Experiment 3).** Force-feeding of the green tea extract alone significantly suppressed the LPS + GalN-induced enhancement of plasma ALT and AST activities in a dose-dependent manner (Fig. 3). The maximum dose, 1.2 g/kg body, corresponded to one third to one half the ingestion of green tea extract per day from the green tea-supplemented (30 g/kg) diet.

**FIGURE 3** Effects of single forced-feeding of graded levels of green tea extract on lipopolysaccharide-induced enhancement of plasma alanine aminotransferase (A) and aspartate aminotransferase (B) activities in d-galactosamine-sensitized rats (Experiment 3). Normal rats were injected with saline. The column and its bar represent the mean value and SEM, respectively, for 5 (normal) or 8 (the other groups) rats. Values with different letters are significantly different at \( P < 0.05 \). ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight gain g/14 d</th>
<th>Food intake</th>
<th>Plasma enzyme activity (( \mu \text{mol/(min} \cdot \text{L}) )</th>
<th>alphabetical number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Saline)</td>
<td>70 ± 2a</td>
<td>202 ± 4a</td>
<td>17 ± 1c</td>
<td>74 ± 3c</td>
</tr>
<tr>
<td>Control (LPS + GalN)</td>
<td>68 ± 2a</td>
<td>198 ± 3a</td>
<td>3859 ± 259a</td>
<td>12,440 ± 529a</td>
</tr>
<tr>
<td>+ Green tea (LPS + GalN)</td>
<td>52 ± 2b</td>
<td>182 ± 4b</td>
<td>1312 ± 79b</td>
<td>3608 ± 357b</td>
</tr>
</tbody>
</table>

\(^1\) Values are mean ± SEM, \( n = 5 \) (normal) or 9 (the other groups).

\(^2\) Values in a column with different superscript letters are significantly different at \( P < 0.05 \). ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Time-dependent effect of green tea (Experiment 4). Dietary supplementation with green tea extract at 35 g/kg depressed the growth and food intake of rats compared with the control group (body weight gain, 70.1 ± 0.8 g for control group; 50.2 ± 0.8 g/14 d for + tea group, n = 50 each, P < 0.001; food intake, 197 ± 2 for control group; 177 ± 1 g/14 d for + tea group, n = 50, P < 0.001). In this experiment, time-dependent changes in plasma enzyme activities and cytokine concentrations were measured up to 24 h after the injection of LPS + GalN. Plasma ALT and AST activities were increased by LPS + GalN injection in control rats with maximum values at 24 h, and these increases were significantly suppressed in the green tea–fed rats (Fig. 4). Injection of LPS + GalN markedly increased plasma concentrations of all the cytokines measured with different peak times (Figs. 5 and 6). Green tea supplementation did not suppress the peak concentrations of TNF-α and MIP-2 1 h after the injection of LPS + GalN, although it slightly accelerated the decline of these cytokine concentrations at 3 h or later (Fig. 5 A, B). In contrast, plasma concentrations of IL-6, IFN-γ, IL-1β, IL-2, IL-4 and IL-10 were significantly suppressed by dietary green tea.

DNA ladder and DNA fragmentation were measured as markers of the apoptosis of liver cells. Injection of LPS + GalN caused a representative ladder pattern of liver oligonucleosomal DNA on agarose gel electrophoresis in control rats, and this DNA ladder was effectively suppressed by dietary green tea extract (Fig. 7). This was confirmed by the quantitative determination of DNA fragmentation. In the control rats, injection of LPS + GalN markedly increased the DNA fragmentation of liver cells (Fig. 8). This time-dependent change in DNA fragmentation was apparently faster than those of plasma ALT and AST activities shown in Figure 4. Increases in DNA fragmentation at 6, 12 and 24 h were significantly suppressed by dietary supplementation with green tea extract.

Effect of dietary caffeine (Experiment 5). Dietary supplementation with caffeine (2 g/kg) depressed growth and food intake of rats (Table 2). The LPS + GalN–induced increases in plasma ALT and AST activities, measured 8 h after the injection of LPS + GalN, were significantly suppressed by dietary green tea extract.

**FIGURE 4** Effects of dietary supplementation with green tea extract on lipopolysaccharide-induced enhancement of plasma alanine aminotransferase (A) and aspartate aminotransferase (B) activities in d-galactosamine-sensitized rats (Experiment 4). The circle and its bar represents mean ± SEM for 5 (0 h) or 8 to 10 (the other time points) rats. The asterisk indicates a significant difference between the two groups at the same time points, P < 0.05. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GalN, d-galactosamine; LPS, lipopolysaccharide.

**FIGURE 5** Effects of dietary supplementation with green tea extract on lipopolysaccharide-induced enhancement of plasma tumor necrosis factor-α (A), macrophage inflammatory protein-2 (B), interleukin-6 (C) and interferon-γ (D) in d-galactosamine–sensitized rats (Experiment 4). IFN, interferon; IL, interleukin; TNF, tumor necrosis factor. See Figure 4 for other details.

**FIGURE 6** Effect of dietary supplementation with green tea extract on lipopolysaccharide-induced enhancement of plasma interleukin-4 (A), interleukin-1β (B), interleukin-2 (C) and interleukin-10 (D) in d-galactosamine-sensitized rats (Experiment 4). See Figure 4 for other details.
injection of the drugs, were significantly suppressed by dietary supplementation with caffeine. On the basis of the results of Experiment 4, plasma concentrations of TNF-α and MIP-2 were measured 1 h after the injection of the drugs; concentrations of IL-1β, IL-6, IL-10 and IFN-γ were measured 8 h after the injection of drugs. Plasma concentrations of most of the cytokines measured, except for MIP-2, were significantly lower in caffeine-fed rats than in control rats. In contrast to the effect of green tea observed in Experiment 4, caffeine significantly suppressed the LPS + GalN–induced increase in plasma TNF-α concentration.

**Effect of green tea on tumor necrosis factor-α-induced liver injury (Experiment 6).** Intravenous injection of TNF-α significantly increased plasma ALT and AST activities and hepatic DNA fragmentation in GalN-sensitized rats (Table 3). The increases in these enzyme activities and DNA fragmentation were significantly suppressed by dietary supplementation with green tea extract.

**DISCUSSION**

We reported previously that liver injury induced by GalN alone could be suppressed effectively by dietary green tea (Sugiyama et al. 1998, 1999). The major finding of this study was that green tea also had a protective effect against LPS + GalN–induced liver injury in rats when fed to rats for 14 d and when force-fed once before injection of the drugs, indicating that the effect of green tea is elicited quickly. Of the five fractions derived from green tea, the caffeine-containing fraction (fraction I) had the strongest effect (Fig. 2) and the effect of green tea was reproduced by authentic caffeine (Table 2), suggesting that the protective effect of green tea against LPS + GalN–induced liver injury might be attributable mainly to caffeine. Because growth and food intake of rats were depressed by dietary supplementation with either fraction
Effects of dietary supplementation with green tea extract (35 g/kg) on tumor necrosis factor-α-induced enhancement of plasma enzyme activities and DNA fragmentation of liver cells in β-galactosamine-sensitized rats (Experiment 6)\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma enzyme activity</th>
<th>DNA fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (μmol/min·L)</td>
<td>AST (μmol/min·L)</td>
</tr>
<tr>
<td>Normal</td>
<td>19 ± 2\textsuperscript{c}</td>
<td>67 ± 4\textsuperscript{c}</td>
</tr>
<tr>
<td>Control</td>
<td>842 ± 44\textsuperscript{a}</td>
<td>1897 ± 72\textsuperscript{a}</td>
</tr>
<tr>
<td>Green tea</td>
<td>304 ± 30\textsuperscript{a}</td>
<td>680 ± 38\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Values are mean ± SEM, n = 5 (normal) or 7 (the other groups) rats.
\textsuperscript{2} Values in a column with different superscript letters are significantly different at P < 0.05. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

1 or caffeine, the effects of green tea on these variables could also be ascribed to caffeine. However, it is unlikely that the protective effect of caffeine against LPS + GalN–induced liver injury was associated with the depressions of growth and food intake per se because there was no significant effect of final body weight or body weight gain on the plasma enzyme activities when analyzed by analysis of covariance. Furthermore, green tea had a strong effect even when it was force-fed to rats once before injection of the drugs, a situation in which the effects of growth and food intake could be ignored. Consistent with the results presented here, Ueda and Yamazaki (1997) reported that green tea extract or caffeine could suppress systemic inflammation induced by muramyl dipeptide derivative and OK-432, a bacterial application, in mice. These findings may be indirect evidence that green tea or caffeine exhibits a hepatoprotective effect by suppressing inflammation. In contrast, Yang et al. (1998) demonstrated that the enhanced mortality and serum TNF-α concentration induced by a large amount of LPS alone (40 mg/kg body) could be suppressed by single force-feeding of tea polyphenols (catechins) in mice. In this study also, fraction II, which contained tea catechins as exclusively major constituents, slightly but significantly suppressed LPS + GalN–induced liver injury. However, it should be noted that the effect of tea catechins was considerably weaker than that of caffeine at least in this LPS + GalN–induced liver injury model.

TNF-α is synthesized and released by macrophages including Kupffer cells, the resident macrophages in the liver, in response to stimulation by LPS and plays a critical role in LPS + GalN–induced acute liver injury or hepatitis (Bradham et al. 1998). Therefore, one of the objectives of this study was to determine whether the protective effect of green tea against LPS + GalN–induced liver injury can be accounted for by the suppression of TNF-α release from macrophages. For this purpose, we investigated the time-dependent effects of dietary green tea on plasma concentrations of eight kinds of cytokines, including TNF-α. Of these cytokines, TNF-α, IL-1β, IL-6 and MIP-2 (a murine functional homologue of human IL-8) are produced mainly by macrophages, whereas IL-2, IL-4, IL-10 and IFN-γ are produced mainly by T lymphocytes. Unexpectedly, green tea extract did not suppress plasma TNF-α concentration at the peak time 1 h after the injection of LPS + GalN, although green tea suppressed LPS-induced increases in plasma concentrations of IL-1β, IL-2, IL-4, IL-6, IL-10 and IFN-γ. Therefore, the results suggest that the protective effect of green tea against LPS + GalN–induced liver injury cannot be ascribed to the suppression of TNF-α release from macrophages into the circulation, at least under the conditions used. In contrast, green tea suppressed TNF-α-induced liver injury in GalN-sensitized rats (Table 3), suggesting that the protective effect of green tea against LPS + GalN–induced liver injury might be elicited through the inhibited action of TNF-α rather than the decreased release of TNF-α. The results also indicate that the effect of green tea on macrophage-derived cytokines was selective because plasma concentrations of IL-1β and IL-6 were significantly suppressed by dietary green tea, whereas TNF-α and MIP-2 concentrations were unaffected. In contrast, green tea seemed to suppress the LPS-associated activation of T lymphocytes because plasma concentrations of T-cell–derived cytokines were all suppressed by green tea.

Unlike green tea extract, caffeine significantly suppressed LPS + GalN–induced enhancement of plasma TNF-α concentration (Table 2). In another series of experiments, we also found that LPS + GalN–induced enhancement of plasma TNF-α concentration could be suppressed effectively by intra-peritoneal injection of caffeine (100 mg/kg) 1.5 h before LPS + GalN injection; TNF-α concentrations 1 h after injection of drugs for normal, control and +caffeine rats were 7 ± 2, 9900 ± 1200 and 518 ± 46 ng/L, respectively. Therefore, it is likely that caffeine protects rats from LPS + GalN–induced liver injury at least in part through suppressed TNF-α release. Ueda and Yamazaki (1997) reported that the chloroform-soluble fraction obtained from green tea, which contains caffeine at a 90% purity, suppressed the enhancement of plasma TNF-α concentration induced by the muramyl dipeptide derivative and OK-432 when the fraction was force-fed alone to mice, whereas the other water-soluble fraction stimulated plasma TNF-α concentration. These results suggest that green tea contains both TNF-α-decreasing and TNF-α-increasing constituents, and that caffeine is one of the TNF-α-decreasing constituents of green tea. The findings of this study that green tea extract and caffeine differentially affected the plasma TNF-α concentration support the idea that green tea contains a TNF-α-increasing constituent, although the identity of the constituent is unclear at present.

Caffeine is one of the naturally occurring methylxanthines that exist abundantly in traditional beverages such as tea and coffee. Hence, it might be expected that coffee also might be effective in suppressing liver injury. Actually, we found that coffee had a protective effect against LPS + GalN–induced liver injury when added to the diet and fed to rats for 14 d or force-fed to rats alone before the injection of drugs (unpublished data). Although little information is available concerning the effects of caffeine on liver injury and plasma concentrations of various kinds of cytokines, several reports have shown that the methylxanthine-derivative drugs have significant effects on liver injury and plasma cytokine concentrations. For example, LPS + GalN–induced liver injury can be suppressed effectively by synthetic methylxanthine-derivative drugs, such as pentoxifylline and A802715, in mice (Fischer et al. 1993 Jilg et al. 1996). cAMP may participate in the hepatoprotective effect of methylxanthine-derivative drugs such as pentoxifylline and A802715 because these drugs inhibit phosphodiesterase activity and thereby raise cellular cAMP concentration (Leist et al. 1996, Semmler et al. 1993, Sinha et al. 1995). Actually, exogenous dibutyl cAMP, a stable and permeable cAMP analog, had a protective effect against LPS-
induced liver injury in Propionibacterium acnes–treated mice (Arai et al. 1995, Taguchi et al. 1999). Exogenous dibutyryl cAMP significantly increased circulating IL-10 concentration, an immunosuppressive cytokine, with a concomitant decrease in TNF-α concentration in LPS-treated mice, suggesting that IL-10 may be involved in the inhibition of LPS-induced liver injury by dibutyryl cAMP (Arai et al. 1995). Furthermore, Jilg et al. (1996) demonstrated that A802715 suppressed the enhancement of plasma concentrations of TNF-α, IFN-γ, IL-1 and granulocyte colony–stimulating factor in mice injected with a high level of LPS alone (3 mg/kg), whereas it inversely increased the plasma concentrations of IL-6 and IL-10. On the basis of these results, they also pointed out that increased IL-10 participates in the prevention of systemic endotoxemia by the methylxanthine-derivative drug. In contrast to those findings, this study demonstrated that green tea extract or caffeine significantly suppressed the LPS + GalN–induced enhancement of plasma IL-10 concentration, indicating that the hepatoprotective effect of green tea or caffeine could not be ascribed to an increment of circulating IL-10 in the LPS + GalN–induced liver injury model. These contrasting results may be due to the following different experimental conditions: 1) difference in drugs used, a high level of LPS alone vs. a low level of LPS + GalN, 2) difference in methylxanthines used, synthetic methylxanthine-derivative drug A802715 vs. natural methylxanthine caffeine; or 3) difference in animal species used, mice vs. rats.

LPS is thought to induce the apoptosis of liver cells through the action of TNF-α in GalN-sensitized mice (Leist et al. 1995). Consistent with this, we demonstrated that TNF-α + GalN, as well as LPS + GalN, induced the apoptosis of liver cells in rats, as evidenced by the fact that liver DNA exhibited a representative ladder on agarose gel electrophoresis and that increase in DNA fragmentation preceded the increase in plasma ALT and AST activities. It has been demonstrated that apoptosis precedes the necrosis of liver cells (Leist et al. 1995), and that the prevention of liver cell apoptosis, e.g., by inhibitors for caspases downstream of TNF receptor 1, was an effective means suppressing LPS- or TNF-α–induced liver injury in GalN-sensitized mice or rat hepatocytes (Hamada et al. 1999, Jaeschke et al. 1998). Such evidence indicates that liver cell apoptosis is an essential process for liver injury, at least LPS- or TNF-α–induced liver injury. Unlike necrosis, apoptosis has been considered to be a noninflammatory death of cells. Also, in liver injury induced by LPS, the apoptosis of liver cells per se is not the direct cause of liver injury; rather, infiltrating neutrophils (polymorphonuclear leukocytes) are thought to initiate parenchymal cell injury and necrosis (Jaeschke et al. 1991, Leist et al. 1995). Recent reports have shown that excessive apoptosis of hepatic parenchymal cells represents an important signal for the transmigration of primed neutrophils sequestered in sinusoids during LPS-induced liver injury (Lawson et al. 1998). The important finding of this study is that green tea suppressed the apoptosis of liver cells induced either by LPS or TNF-α in GalN-sensitized rats. Another part of our study showed that caffeine could effectively prevent LPS- or TNF-α–induced apoptosis of liver cells in rats and mice (unpublished data), suggesting that the suppressive effect of green tea on liver cell apoptosis is attributable mainly to caffeine. Thus, it is likely that caffeine exerts its protective effect against LPS + GalN–induced liver injury by both the inhibition of TNF-α release from macrophages and the inhibition of TNF-α–induced apoptosis of liver cells, although the latter mechanism seems to be the predominant effect of green tea. In humans, liver injury or hepatitis is caused by viruses, chemicals, alcohol and autoimmune diseases. It has been demonstrated that TNF-α participates in liver injury induced by virus (Ando et al. 1997), alcohol (Yin et al. 1999) and immune disease (Gantner et al. 1995). Therefore, it may be possible that caffeine-containing beverages prevent or alleviate these types of liver injury, although further evidence is required to confirm this. The detailed mechanism by which caffeine inhibits the apoptosis of liver cells also remains to be elucidated.

LITERATURE CITED


