**Note**

Suppressive Effect of Caffeine on Hepatitis and Apoptosis Induced by Tumor Necrosis Factor-\(\alpha\), but Not by the Anti-Fas Antibody, in Mice

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Tumor necrosis factor (TNF-\(\alpha\))-induced hepatitis and apoptosis, as respectively assessed by serum enzyme activities and hepatic DNA fragmentation were effectively suppressed by a single force-feeding of caffeine (100 mg/kg) 1.5 h before injecting the drug. In contrast, caffeine had no significant effect on anti-Fas antibody-induced hepatitis and apoptosis. These results suggest that caffeine differentially affected TNF-\(\alpha\) receptor- and Fas-mediated hepatitis and apoptosis.

**Key words:** caffeine; tumor necrosis factor-\(\alpha\); anti-Fas antibody; hepatitis; apoptosis

It is widely recognized that tea has a variety of biological effects. We have reported that green tea or its constituents, e.g., flavonoid glycosides, theanine, and soluble dietary fibers, could protect rats from D-galactosamine (GalN)-induced liver injury or hepatitis.\(^1\)\(^\text{a}\) Furthermore, we found that lipopolysaccharide (LPS)-induced hepatitis was also suppressed by dietary supplementation with a green tea extract in GalN-sensitized rats, and that this effect of green tea was mainly attributable to the caffeine content (unpublished results). Hepatitis induced by LPS + GalN is one of the most frequently used hepatitis models. Tumor necrosis factor (TNF-\(\alpha\)), which is released mainly from macrophages in response to LPS, plays a central role in the pathogenesis of LPS-induced hepatitis.\(^2\) TNF-\(\alpha\) induces apoptosis of hepatocytes through signal transduction which is initiated by the binding of TNF-\(\alpha\) to TNF receptor 1 and this apoptosis is believed to act as a primary initiating step of hepatitis.\(^3\) Apoptosis of hepatocytes is also induced by the Fas ligand, one of the mediators of virus-induced hepatitis.\(^4\) It is known that the TNF receptor and Fas, the receptor of the Fas ligand, belong to the same family, and that some caspases, e.g., caspases 1 and 3, are commonly involved in TNF receptor- and Fas-mediated apoptosis.\(^5\) These facts prompted us to investigate whether TNF-\(\alpha\) and Fas ligand-induced hepatitis and apoptosis could be modified by green tea or its active constituents in the same manner. We investigated in this present study the in vivo effects of caffeine on hepatitis and apoptosis induced by TNF-\(\alpha\) and the anti-Fas antibody, an agonist for Fas, in mice. The results clearly show that caffeine suppressed TNF-\(\alpha\)-induced hepatitis and apoptosis, while it failed to suppress anti-Fas antibody-induced hepatitis and apoptosis.

Caffeine and recombinant murine TNF-\(\alpha\) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.) and Funakoshi (Tokyo, Japan), respectively. The monoclonal hamster anti-mouse Fas antibody, Jo-2, was purchased from PharMingen (San Diego, CA, U.S.A.). Six-week-old ddby mice were purchased from Japan SLC (Hamamatsu, Japan) and kept in an isolated room at a controlled temperature (23–25°C) and ambient humidity (50–60%). Lighting was provided on a 12-h light-dark cycle (lights on from 0700 to 1900 h). The animals were acclimatized to the facility for 5 to 7 days and given free access to a commercial diet (type MF; Oriental Yeast, Tokyo, Japan).

Three separate in vivo experiments were conducted in this study. In experiment 1, mice weighing 37.1 ± 0.2 g were injected intraperitoneally with the anti-Fas antibody (3.5, 5.0, 6.5, 8.0 or 10 \(\mu\)g/mouse) or the vehicle (PBS) alone to measure the dose-dependent effect of the antibody, since the anti-Fas antibody exhibits a severe lethal effect. In experiment 2, mice weighing 36.7 ± 0.2 g were force-fed with caffeine (100 mg/kg of body weight) or water by stomach tube, before being given an intraperitoneal injection of recombinant murine TNF-\(\alpha\) (15 \(\mu\)g/kg of body weight) plus GalN (700 mg/kg of body weight) or saline 1.5 h after administering the caffeine. In experiment 3, mice weighing 38.2 ± 0.2 g were force-fed with caffeine or water, before being given an intraperitoneal injection of the anti-Fas antibody (6.5 \(\mu\)g/mouse) or PBS 1.5 h after administering the caffeine. The mice were killed by decapitation to obtain the blood and liver 8 or 6 h after being injected with TNF-\(\alpha\) or the anti-Fas antibody, respectively. The blood serum was separated from the whole.

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blood by centrifugation at 2,000×g for 20 min at 4°C. The serum and liver were stored at −80°C until needed for analyses. The experimental design of this study was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture at Shizuoka University.

The magnitude of hepatitis was estimated by the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. These enzyme activities were measured with a kit (Wako Pure Chemical, Osaka, Japan).

Apoptosis of the liver cells was visualized by a ladder analysis of liver DNA and quantified by measuring the DNA fragmentation. The hepatic DNA fragmentation was respectively detected and quantified by agarose gel electrophoresis and DNA ELISA, according to the methods of Leist et al. and Hase et al. with slight modifications. In brief, frozen liver was homogenized in 4 volumes (volume/weight) of ice-cold PBS containing 10 mm EDTA. The homogenate was centrifuged at 13,000×g for 20 min at 4°C, and cytosolic DNA was prepared from the supernatant (0.3 ml) by extracting with an equal volume of phenol-chloroform (1:1, volume/weight), precipitating in ethanol, and subsequently treating with 20 μg/ml of ribonuclease A (Sigma) for 30 min at 37°C. The resulting DNA was electrophoresed on 1.8% agarose gel. An aliquot of the supernatant of the liver homogenate was diluted and subjected to a direct analysis of oligonucleosome-bound DNA with an ELISA kit (Boehringer Mannheim, Mannheim, Germany). Data were analyzed by a one-way analysis of variance, and the difference between means was tested at p<0.05 by using Duncan’s multiple-range test.

The activities of serum ALT and AST increased in response to the injection of the anti-Fas antibody in a dose-dependent manner (Fig. 1). In this experiment, the anti-Fas antibody did not cause death in any of the mice up to a dose of 6.5 μg/mouse, but it brought about 40% and 70% mortality at doses of 8 and 10 μg/mouse, respectively, during the 6-h period after injecting the antibody. Based on these results, we decided to use the anti-Fas antibody at a dose level of 6.5 μg/mouse in experiment 3. On the other hand, the dose level of TNF-α (15 μg/kg) was determined according to our another series of experiments. The injection of TNF-α in combination with GalN brought about higher levels of serum ALT and AST activities than those in normal mice (Table 1). Pretreatment of the mice with caffeine markedly suppressed the enhancement of serum enzyme activities, indicating that caffeine effectively protected the mice from TNF-α-induced hepatitis. Caffeine was also found to suppress the TNF-α-induced increase in DNA fragmentation in the liver. The decrease in DNA fragmentation by caffeine was also observed by agarose gel electrophoresis of cytosolic DNA (Fig. 2). Surprisingly, the caffeine pretreatment increased the mortality of the mice due to the anti-Fas antibody. Furthermore, the anti-Fas antibody-induced enhancement of serum ALT and AST activities in the surviving mice and hepatic DNA fragmentation were not suppressed by the caffeine pretreatment, indicating that caffeine had no protective effect against anti-Fas antibody-induced hepatitis and apoptosis.

### Table 1. Effect of Caffeine on Tumor Necrosis Factor-α and Anti-Fas Antibody-induced Enhancement of the Plasma Enzyme Activities and DNA Fragmentation of Liver Cells in Mice

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Serum enzyme activity</th>
<th>DNA fragmentation</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ALT (μmol/min/l)</td>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Water</td>
<td>PBS</td>
<td>9 ± 1</td>
<td>54 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100 ± 11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>TNF-α</td>
<td>1505 ± 227&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2420 ± 513&lt;sup&gt;c&lt;/sup&gt;</td>
<td>451 ± 14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>TNF-α</td>
<td>265 ± 63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>448 ± 75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>162 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.</td>
<td>Water</td>
<td>PBS</td>
<td>10 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>Anti-Fas Ab</td>
<td>1857 ± 374&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2755 ± 362&lt;sup&gt;a&lt;/sup&gt;</td>
<td>509 ± 31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>Anti-Fas Ab</td>
<td>2609 ± 282&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3814 ± 426&lt;sup&gt;a&lt;/sup&gt;</td>
<td>583 ± 32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Each value is the mean ± SEM for the number of surviving mice. In each experiment, values with no common superscript letters are significantly different at p<0.05. ALT, alanine aminotransferase; AST, aspartate aminotransferase; TNF-α, tumor necrosis factor-α; Ab, antibody.
The results clearly demonstrate that caffeine differentially affected TNF-α and anti-Fas antibody-induced hepatitis and apoptosis of the liver cells in mice. The finding that caffeine could markedly suppress TNF-α-induced hepatitis and apoptosis appears to help understand the mechanism by which caffeine-containing beverages suppress LPS-induced hepatitis, since TNF-α is thought to play a central role in the pathogenesis of LPS-induced hepatitis. In another series of experiments, we found that an intraperitoneal injection of caffeine (100 mg/kg) suppressed the LPS-induced increase in serum TNF-α concentration in rats when serum samples were taken 1 h after injecting LPS + GaIN; the TNF-α concentrations for normal, control, and + caffeine rats were 7 ± 2, 9900 ± 1200, and 518 ± 46 pg/ml, respectively. It is therefore likely that caffeine suppressed LPS-induced hepatitis by two mechanisms, i.e., suppression of TNF-α production and suppression of TNF-α-induced apoptosis of liver cells.

Although the detailed mechanism by which caffeine suppressed LPS- or TNF-α-induced hepatitis is not known, it seems possible that it elicited its response through the enhanced cAMP concentration in the liver, because it has been shown that synthetic methylxanthine-derivative drugs such as pentoxifylline (3,7-dimethyl-1-(5-oxohexyl)-xanthine) and A802715 (1-(5-hydroxy-5-methyl)-hexyl-3-methyl-7-propylxanthine) inhibited LPS- or TNF-α-induced hepatitis in addition to inhibiting the phosphodiesterase activity. This may also be the case for caffeine, since caffeine is one of the naturally occurring inhibitors of phosphodiesterase activity.

Another finding from this study is that caffeine failed to suppress anti-Fas antibody-induced hepatitis and apoptosis. This is an unexpected result, since Okamoto has recently reported that pentoxifylline inhibited anti-Fas antibody-induced hepatitis at doses of 10 and 100 mg/kg of body weight in mice. He also showed that pentoxifylline could not suppress the anti-Fas antibody-induced increase in CPP32 (caspase-3)-like activity in the liver. Based on these observations, he deduced that pentoxifylline might have inhibited anti-Fas antibody-induced hepatitis by affecting a reaction downstream of the CPP32-like protease activation. In contrast, the result of the present study demonstrate that caffeine rather exacerbated the effect of the anti-Fas antibody, judging from the effect on the mortality of the mice. The mechanism for this effect for caffeine is currently unclear.

Several recent reports have demonstrated that anti-Fas antibody-induced hepatitis could be significantly suppressed by naturally occurring compounds such as glycyrhrizin and genipin in mice. On the other hand, in human HepG2 cells, glycyrhrizin did not inhibit anti-Fas antibody-induced apoptosis, while it significantly inhibited TNF-α-induced apoptosis. The result of the present study support the notion that TNF-α and anti-Fas antibody-induced apoptosis of hepatocytes and resulting hepatitis can be differentially affected by hepatoprotective compounds. This may be correlated, at least in part, to the fact that signal transduction via the TNF receptor and Fas is different. For instance, the hepatotoxicity of TNF-α is thought to be associated with an apoptotic mechanism that manifests itself under the condition of arrested transcription and functional translation, whereas Fas-mediated liver injury does not require any transcriptional arrest. It remains to be clarified whether tea constituents other than caffeine have a protective effect against Fas-mediated hepatitis.

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References


