The anti-fibrotic effect of green tea with a high catechin content in the galactosamine-injured rat liver

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ABSTRACT
Previously, we reported that the oral administration of green tea rich in catechins restored levels of several biomarkers increasing in galactosamine-treated rats to nearly control values. These biomarkers included serum transaminase activities, serum concentrations of tumor necrosis factor-α and interleukin 1-β, and the hepatic mRNA expression of these inflammatory cytokines. In the present study, we examined possible anti-fibrotic effects of green tea in galactosamine-induced hepatitis. The results of the reverse transcription and polymerase chain reaction indicated that the increase in gene expression of the α1 chain of collagen type 1 and transforming growth factor β-1 in the injured liver 24 h post-injection of galactosamine was suppressed by the administration of green tea. Masson’s trichrome staining demonstrated that the extent of fibrogenesis after 14 days was greater in the galactosamine-injured livers not treated with green tea than the treated ones. These results suggest that the drinking of green tea with a high catechin content may help to prevent and/or attenuate the development of fibrosis in hepatitis.

Galactosamine is known to induce hepatic injury in rats that is similar in pathophysiology to viral hepatitis and drug-induced hepatitis in humans (11, 14). Hepatic fibrosis is a common response to chronic liver injury from many causes including alcohol and viral infection (6). Progressive fibrosis eventually leads to cirrhosis which is often associated with a high risk of hepatocellular carcinoma (9, 10). In rats, multiple injections of D-galactosamine induce liver fibrosis and cirrhosis (12) and galactosamine hepatitis caused by a single dose of the drug may reflect an early phase of liver fibrosis.

Previously, we showed that the oral administration of green tea rich in catechins restored levels of several biomarkers in rats treated with a single dose of galactosamine nearly to the control values (1). These biomarkers included serum transaminase activities, serum concentrations of tumor necrosis factor-α and interleukin 1-β, and the hepatic mRNA expression of these inflammatory cytokines. Since only limited information is available on the effects of green tea or its components in liver fibrosis, we examined the possible anti-fibrotic effect of green tea in galactosamine-induced hepatitis.

MATERIALS AND METHODS

Materials. Healthya green tea, a commercial green tea drink produced by Kao Ltd. (Tokyo, Japan), was purchased from a convenience store. Healthya green tea has minimum contents of 540 mg of catechins and 80 mg of caffeine per 350 mL. D-galactosamine was obtained from Wako Pure Chemical Industries

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Animal experimental design. Ethical approval for the study was obtained from the Committee for Animal Experimentation of the University of Shizuoka. Male Sprague-Dawley rats (7 week old, 210–230 g) were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). They were housed individually and maintained with standard solid laboratory chow MF (Oriental Yeast Co. Ltd., Tokyo, Japan). The animals were divided into four groups of 3 for each: the control group (Group I) and the galactosamine-treated group (Group II) freely received water, whereas the two other groups freely received Healthya green tea during the experimental periods with (Group III) or without the galactosamine-treatment (Group IV). Galactosamine was administered by intraperitoneal injection (500 mg/kg) (1, 18). The other groups received an intraperitoneal injection of saline (5 mL/kg). After 24 h and 14 days, the animals were anesthetized with ether, and blood samples were collected from the inferior vena cava. The serum was separated and kept frozen at −85°C prior to use. Liver samples for reverse transcription-polymerase chain reaction (RT-PCR) were kept at −85°C in RNAlater (Takara Bio Co. Ltd., Tokyo, Japan). A portion of each liver sample was fixed in 10% phosphate-buffered formalin for histological examination.

Histology. Fixed liver samples were embedded in paraffin. Three-micrometer sections were cut and subjected to Masson’s trichrome staining for an evaluation of liver fibrosis (15).

Serum transaminase activity. Alanine transaminase and aspartate transaminase levels were determined using commercial kits, Transaminase CII Test Wako (GOT and GPT) (Wako Pure Chemical Industries Ltd.).

RT-PCR for collagen and transforming growth factor (TGF)-β1. Total RNA was extracted from the liver and mRNA was prepared using a QIAamp RNA Blood Mini Kit (Qiagen Ltd., Tokyo, Japan) according to the manufacturer’s directions. To prevent possible contamination, samples were treated with deoxyribonuclease (RT-grade, Wako Pure Chemical Industries Ltd.) as recommended by the manufacturer (1, 19). RT-PCR was performed as described previously (1, 19). Primers used were: 5’-CAACTGGCAACCTCAAGAAG-3’ and 5’-GATTGGATGGAGGAGGTGA-3’ for the α1 chain of collagen type 1 (collagen α1(I), GenBank accession no. XM_213440), 5’-CAATTCCTGGCGTTACCTTG-3’ and 5’-CAGTGAGCACTGAAGCGAAA-3’ for TGF-β1 (NM_021578), 5’-ACATCATCCCTGCATCCACT-3’ and 5’-TCTGGGATGGAAATTGTGAGG-3’ for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (XM_573896), and 5’-ACCGTGAAGAGATGACCCA-3’ and 5’-AGGAAGGAAGGCCCTGGAAAGA-3’ for β-actin (NM_031144). Amplified DNA was subjected to electrophoresis in 2% agarose, stained with SYBR Green I (Molecular Probes Ltd., Eugene, Oregon, USA), and imaged and calculated using a FluorImager (Molecular Dynamics Tokyo Ltd., Tokyo, Japan) as described previously (1, 19). Expected sizes of amplified DNA were 278 bp, 143 bp, 522 bp, and 457 bp for collagen α1(I), TGF-β1, GAPDH, and β-actin, respectively.

RESULTS

Weight gain
Each rat in Groups III and IV consumed about 50 mL of Healthya green tea per day which was comparable with the volume of water consumed in Groups I and II. No significant differences in weight gain were observed among the 4 groups under the conditions used (data not shown).

Histology
Rat liver sections were subjected to histological examination with Masson’s trichrome staining 24 h (Fig. 1) and 14 days (Fig. 2) following the galactosamine treatment. As shown in Fig. 1, a number of necrotic areas with infiltration by inflammatory cells in the lobules were observed after 24 h in the galactosamine-treated group (Group II), but the number was greatly reduced in Group III, suggesting that the drinking of Healthya green tea attenuated the liver injury caused by galactosamine. Group IV given the Healthya beverage showed no differences from the control group (Group I).

Fourteen days after the injection of galactosamine, the liver from Group II showed significant positive staining with aniline blue dye, indicating that significant amounts of collagen fibers still existed (Fig. 2). Group III showed fibrogenic features weaker than those of Group II, and no fibrogenesis was observed in other groups (Fig. 2).

Serum transaminase activities
In a previous paper (1), we reported that highly elevated levels of alanine transaminase activity and as-
Fig. 1  Galactosamine-induced liver injury after 24 h and effects of Healthya green tea (Masson’s trichrome stain). Untreated rats (Group I) served as a control. Rats treated with galactosamine (500 mg/kg) (Group II) showed scattered areas of necrosis with infiltration by inflammatory cells in the lobules and tea intake caused a significant reduction in galactosamine-mediated injury (Group III). Administration of tea alone caused no change in histology (Group IV) as compared with the control (Group I). X100

Fig. 2  Galactosamine-induced liver injury after 14 days and effects of Healthya green tea (Masson’s trichrome stain). Liver sections were examined 14 days after a single injection of galactosamine. Untreated rats (Group I) served as a control. Rats treated with galactosamine (500 mg/kg) (Group II) showed positive staining for fibrosis and tea intake caused a significant reduction in this stain (Group III). Administration of tea alone caused no change in histology (Group IV) as compared with the control (Group I). X100
partate transaminase activity were observed after 24 h in the galactosamine-treated group and that oral administration of Healthya green tea reduced the 24-h effect of galactosamine (Table 1). After 14 days, no significant differences were found among the four experimental groups in these activities (Table 1), suggesting no direct damages of hepatocytes.

**RT-PCR**

The results of the RT-PCR showed that intraperitoneal injection of galactosamine caused the enhanced expression of collagen α1 (I) mRNA (Fig. 3) and TGF-β1 mRNA (Fig. 4) in the rat liver after 24 h. Oral administration of Healthya green tea had the effect of lowering the mRNA levels raised by galactosamine (Figs. 3 and 4). Oral administration of the tea itself had no significant effect on these levels (Figs. 3 and 4).

When the liver specimens were examined after 14 days, no significant differences were observed in these mRNA levels (Figs. 5 and 6).

**DISCUSSION**

In the present study, we observed an elevation in the hepatic mRNA levels of collagen α1 (I) and TGF-β1 24 h after a single intraperitoneal injection of galactosamine in rats. These effects were attenuated by oral administration of the catechin-rich tea drink Healthya green tea. The intake of the catechin-rich tea drink by itself had no effect on the hepatic expression of the mRNA of these proteins.

Histological examination showed that 14 days after the injection of galactosamine, significant fibro-

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**Table 1** Effects of tea intake on the serum level of aminotransferase activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Alanine aminotransferase</th>
<th>Aspartate aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h*</td>
<td>14 days</td>
</tr>
<tr>
<td>I</td>
<td>39 ± 12.0</td>
<td>30.7 ± 0.6</td>
</tr>
<tr>
<td>II</td>
<td>1,505 ± 38.0</td>
<td>30.3 ± 7.1</td>
</tr>
<tr>
<td>III</td>
<td>405 ± 142.9</td>
<td>28.3 ± 9.2</td>
</tr>
<tr>
<td>IV</td>
<td>38 ± 8.0</td>
<td>26.7 ± 3.8</td>
</tr>
</tbody>
</table>

Values indicate the mean ± SEM as expressed in IU/L from three different determinations.

*Data cited from our previous paper (1).
Anti-fibrotic effect of green tea

The anti-fibrotic effect of green tea was observed in the liver of galactosamine-injured rats. The collagen α1(I) and TGF-β1 mRNA levels were reduced in the green tea-treated group compared to the control. The suppression of TGF-β1 expression by tea constituents at an early stage of hepatic injury may be responsible for the decreased synthesis of collagen, leading to diminished features of fibrosis at a later phase. The injection of galactosamine enhanced fibrogenic activity as determined by measuring the serum levels of collagen type 3 peptide. RNA interference using TGF-β1 siRNA showed an anti-fibrotic effect in the livers injected with carbon tetrachloride with decreased expression of type 1 collagen.

TGF-β1 is the best characterized profibrogenic cytokine and plays a major role in the development of hepatic fibrosis and liver cirrhosis. Indeed, serum and tissue levels of this cytokine can be used to predict progressive liver fibrosis in patients with hepatitis C infection. TGF-β1 mRNA expression correlated closely with the expression of procollagen type I and patients with increased fibrogenic activity as determined by measuring the serum level of collagen type 3 peptide had an increased level of the cytokine. RNA interference using TGF-β1 siRNA showed an anti-fibrotic effect in the livers injected with carbon tetrachloride with decreased expression of type 1 collagen.

In the present study, we found for the first time that the administration of green tea resulted in a decrease in hepatic mRNA levels of collagen and TGF-β1 in the galactosamine-injured liver. Thus, the suppression of TGF-β1 expression by tea constituents at an early stage of hepatic injury may be responsible for the decreased synthesis of collagen, leading to the diminished features of fibrosis at a later phase. However, it is not clear at present what component of the tea is responsible for this effect.

Using a rat model of hepatic fibrosis with alcoholic liver disease, it has been shown that tea polyphenols exhibited anti-fibrotic effects probably through their antioxidative activities. Hepatic stellate...
cells play a central role in hepatic fibrosis (21), and epigallocatechin gallate, the major green tea polyphenol catechin, has been shown to inhibit collagen production in these cells (17). It has also been demonstrated that epigallocatechin gallate inhibits activation of these cells by suppressing Rho signaling, suggesting its therapeutic potential in the setting of liver fibrosis (8). Furthermore, Zhong et al. (22) showed in experimental cholestasis using ligation of the bile duct for 3 weeks that green tea polyphenols decreased rat hepatic injury accompanying an attenuation of increases in the mRNA expression of procollagen α1 (I) and TGF-β1 caused by the cholestasis.

It is, therefore, conceivable that catechins in the tea drink contributed, at least in part, to the in vivo anti-fibrotic effects of tea observed in the present study. These findings suggest that tea-drinking may be useful to prevent hepatic injury and the accompanying fibrosis with a certain kind of etiology.

REFERENCES