Protective Effect of Celosian, an Acidic Polysaccharide, on Chemically and Immunologically Induced Liver Injuries

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Hepatoprotective effect of celosian, an acidic polysaccharide isolated from the water extract of the seed of Celosia argentea, was investigated using chemical and immunological liver injury models. Celosian inhibited the elevation of serum enzyme (GPT, GOT, LDH) and bilirubin levels on carbon tetrachloride (CCL4)-induced liver injuries in rat. In addition, the hepatoprotective effect of celosian was also observed in this model of liver injury by histopathological findings. Moreover, celosian suppressed rises in GPT or mortality on fulminant hepatitis induced by d-galactosamine/lipopolysaccharide (d-GalN/LPS) or Propionibacterium acnes/LPS in mice. These findings suggested that celosian is an active component in protection against chemical and immunological hepatitis and the activity was found to be a dose dependent.

Celosian showed a concentration dependent inhibitory effect on lipid peroxide (LPO) generation in vitro. Though celosian did not reduce the release of tumor necrosis factor-α (TNF-α), it protected against recombinant human TNF-α (rhTNF-α)-induced liver injury in d-galactosamine sensitized mice.

Key words celosian; acidic polysaccharide; Celosia argentea; hepatoprotective effect; TNF-α; lipid peroxidation

Various factors have been reported to induce hepatic injury. In chemically induced liver injury such as by carbon tetrachloride (CCL4), it was reported that the lipid peroxidation of unsaturated fatty acid binding cells and intracellular organ membranes played important roles.1–3 On the other hand, d-galactosamine/lipopolysaccharide (d-GalN/LPS)-induced or heat-killed Propionibacterium acnes/LPS-induced hepatitis is known to immunologically induce liver injury.4,5 In contrast to liver injury caused by CCL4 via lipid peroxidation, this second type of hepatitis does not implicate direct oxidative tissue degradation. It is dependent, rather, on the release of a potent mediator, such as cytokines, from macrophages. In particular, tumor necrosis factor (TNF) is thought to be a terminal mediator in both d-GalN/LPS-induced and P. acnes/LPS-induced liver injuries.6,7

TNF was discovered in 1975, in the serum of BCG-primed and endotoxin-treated animals, as a polypeptide that induces hemorrhagic necrosis of tumors in recipient animals.8 TNF is a multifunctional cytokine that appears to play an important role in the pathogenesis of tissue damage seen in multiple untreated conditions such as septic shock, inflammation, malaria, diabetes mellitus, cachexia and AIDS.9–11 In hepatitis, it was reported that TNF correlated to the liver damage.12 We paid attention to this cytotoxic cytokine in order to elucidate the inhibitory effect of celosian on d-GalN/LPS- or P. acnes/LPS-induced hepatitis.

In the course of our previous searches for hepatoprotective substances in traditional medicines, the water extracts of 12 traditional drugs were tested in liver injury models, and Celosia argentea was finally selected. Celosian, a novel polysaccharide with a potent hepatoprotective activity, was isolated from the seeds of Celosia argentea.13 In this paper, we wish to report details of the hepatoprotective effects of celosian on various experimental liver injuries induced by both chemical and immunological responses.

MATERIALS AND METHODS

Materials Celosian, white powder, [α]D +141.7° (H2O, c = 0.04), a yellow coloration with phenol/sulfuric acid reagent, molecular mass: 190000 (standard dextran), isolated from the seeds of Celosia argentea, using a combination of DEAE Toyopearl 650M column chromatography (4.6 × 30 cm) and Toyopearl HW-60F gel column chromatography (3 × 70 cm). It was composed of molar ratio of sugar components; arabinose: rhamnose: mannose: galactose: galacturonic acid: glucose: fructose: glucuronic acid: arabinol: sorbitol = 32.9: 18.5: 18.3: 11.4: 8.3: 5.7: 2.1: 1.9: 0.8: 0.1. Proteins: 4% (Lowry method).14

Celosian was sterilized by a membrane filter (0.45 μm) before injection into animals, and results of an endotoxin test using Limulus Test Wako (Wako Pure Chemical Industry, Osaka, Japan) were negative (˂0.02 EU/mg).

Details of the isolation and characterization of celosian have already been discussed in our previous paper.13

CCL4 and d-GalN were obtained from Wako. LPS; (E. coli 055: B5) was purchased from Difco Laboratories, U.S.A. An ELISA kit for the determination of serum TNF-α in mice and human recombinant TNF-α (7.69 × 106 IU/mg) was purchased from Genzyme Co., U.S.A. Propionibacterium acnes was provided by Kankosa Co., Ltd., Osaka, Japan.

Animals Male Sprague-Dawley rats, 6 weeks, weighing 150—170 g, were used for the CCL4-induced liver injury model. Male ddY mice, 6 and 4 weeks, weighing 30—32 and 24—26 g were used for d-GalN/LPS- and TNF-α-induced liver injury models, respectively. Male BALB/c mice were used for the P. acnes/LPS-induced liver injury

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model. All animals were purchased from Shizuoka Laboratory Animal Center, Japan, and maintained under a 12 h light/dark cycle in a temperature and humidity controlled room. The animals were fed a laboratory pellet chow (Clea Japan Inc., Tokyo, Japan; protein 24.0%, lipid 3.5%, carbohydrate 60.5%) and given water ad libitum.

**CCL4-Induced Liver Injury** Liver injury was induced by CCl₄ according to the most commonly used method. In each group, 6 or 7 rats were used. After 12 h of fasting, a mixture of CCl₄ in olive oil (1:1) was injected s.c. at a dose of 6 ml/kg. Celosian was administered three times with a dose of 25 mg/kg, i.p., 24, 12 and 1 h before CCl₄ injection. The serum parameters were checked to measure the extent of liver damage on 24 h after CCl₄ injection.

**d-GalN/LPS-Induced Liver Injury** Liver injury was induced by d-GalN/LPS according to the method described by Tiegs et al. In each group, 10 or 11 mice were used. After 12 h of fasting, the mice were injected i.p., 700 mg/kg of d-GalN and 10μg/kg of LPS. Celosian was administered two times at doses of 1, 10 and 50 mg/kg, s.c., 18 and 2 h before d-GalN/LPS injection. The blood glutamic-pyruvic transaminase (GPT) level was examined to measure the extent of liver damage 8 h after d-GalN/LPS injection.

**P. acnes/LPS-Induced Liver Injury** Liver injury was induced by P. acnes/LPS according to the method of Ferluga and Allison. In each group, 12—14 mice were used. Mice were injected i.v. with 1 mg/mouse of heat-treated P. acnes through the tail vein, and on day 7 they were given i.v. 50 μg/mouse of LPS. The survival rate was observed up to 24 h. Celosian was administered two times, at a dose of 10 mg/kg, i.p., 18 and 2 h before the LPS challenge.

**Histopathological Examination** A portion of the median lobe of the liver was removed 24 h after CCl₄ injection in rats. It was fixed in 10% neutralized formalin solution, embedded in paraflin, sectioned, and stained with hematoxin-lisin-eosin (H&E). Liver cell necrosis and fatty change were graded: (−) normal, (+) slight or centrilobular necrosis, (+ +) moderate or midzonal necrosis, (+ + +) severe or peripheral massive necrosis.

**Enzyme Assay** Serum glutamate oxaloacetate transaminase (GOT), GPT and bilirubin levels in rats, and the blood GPT level in mice, were measured by Reflotron S system (Boeringer Mannheim Co., Ltd., Osaka, Japan). Serum l-lactate dehydrogenase (LDH) level in rats was measured with an LDH monotest (Boeringer Mannheim Co., Ltd., Osaka, Japan) by the use of a UV spectrophotometer.

**Lipid Peroxidation Assay** Preparation of rat liver microsomes and lipid peroxide (LPO) generation followed the procedures of Ernst and Nordenbrand. The livers were collected from 12 h fasted rats. Each liver was homogenized in cold 1.15% KCl solution (1 ml/g wet tissue) and centrifuged at 10000 × g for 10 min. The supernatant was further centrifuged at 100000 × g for 60 min. The pellet was suspended in the same volume of fresh 1.15% KCl solution. The protein concentration of the microsomal suspension was determined by the Lowry method using albumin as the standard.

The reaction mixture (1.0 ml) for lipid peroxidation was composed of 0.2 mM ascorbate, 0.01 mM FeSO₄, and various concentrations of celosian and the microsomal suspension (20 μg protein/ml). After incubation at 37°C for 20 min, lipid peroxide in the reaction mixture was measured by the method of Ohkawa et al.

**TNF-α Assay** Hepatitis was induced by d-GalN/LPS as described above. Celosian was administrated s.c. twice at a dose of 50 mg/kg, 18 and 2 h before the d-GalN/LPS challenge. The blood sample in mice was collected at 2 h prior, just before, and 1, 2, 4 h after the d-GalN/LPS injection, and serum TNF-α was determined by an ELISA kit.

**TNF-α-Induced Liver Injury** Liver injury was induced by TNF-α according to the method described by Wendel and Sakaguchi et al. In each group, 10 or 11 mice were used. After 12 h of fasting, the mice were sensitized with 700 mg/kg of d-GalN, i.p., and after 1 h, 4 μg/kg of TNF-α was injected i.v. Celosian was administered s.c., two times at a dose of 50 mg/kg, 18 and 2 h before d-GalN administration. The blood GPT level was examined 8 h after d-GalN administration (7 h after TNF-α injection) to measure the extent of liver damage.

**Statistical Analysis** All values are expressed as mean ± S.E., and were obtained from n number of experiments. The Student's t-test for unpaired observation between the control and experimental samples was carried out for statistical evaluation of a difference. Kaplan-Meier method/generated Wilcoxon test was used for the evaluation of mortality; a p value of 0.05 or less was considered statistically significant.

**RESULTS**

**Effect of Celosian on CC14-Induced Liver Injury in Rats** The results of the hepatoprotective effect of celosian on CC14-induced liver injury in rats are shown in Table 1. In the CC14-treated control, serum GOT, GPT, LDH and bilirubin levels were 1354, 417, 1059 IU/l and 561 μg/dl, respectively, 24 h after CC14 administration. In contrast, in the celosian pretreated group, GOT (203 IU/l), GPT (83.2 IU/l) and LDH (92.9 IU/l) and bilirubin (255 μg/dl) were observed at a near normal level. We also compared the effect of celosian with glycyrhrizin, a clinically used hepatoprotective agent. The measurement of serum GOT, GPT, LDH and bilirubin shown in Table 1 suggested that celosian was more effective than glycyrhrizin. The effect of celosian on histopathological examinations was also evaluated using this model and the results are shown in Table 2 and Fig. 1. The liver tissues of the control group after 24 h CC14 injection were observed to have midzonal or massive necrosis surrounded by a rim of hydropic cells in Kiernan's classic hexagonal lobule. These pathological alternations were reduced by pretreatment using 25 mg/kg of celosian. A histopathological test, as well as serum parameters, suggested that celosian is a very strong hepatoprotective agent for chemically induced hepatitis.

**Effect of Celosian on d-GalN/LPS-Induced Liver Injury in Mice** Furthermore, the hepatoprotective effect of celosian was evaluated in immunological liver injury in mice. The extent of liver damage was expressed in terms
Table 1. Effects of Celosian from Ceiostia argentea and Glycyrhrizin on CCL4-Induced Liver Injury in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>sGOT (U/l)</th>
<th>sGPT (U/l)</th>
<th>sLDH (U/l)</th>
<th>sBilirubin (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>--</td>
<td>7</td>
<td>74 ± 4.9</td>
<td>37 ± 2.5</td>
<td>156 ± 24</td>
<td>N.D.</td>
</tr>
<tr>
<td>CCL4 treated control</td>
<td>--</td>
<td>7</td>
<td>1354 ± 229</td>
<td>417 ± 134</td>
<td>1059 ± 219</td>
<td>561 ± 58</td>
</tr>
<tr>
<td>Glycyrhrizin</td>
<td>100</td>
<td>7</td>
<td>708 ± 124*</td>
<td>170 ± 34*</td>
<td>267 ± 46*</td>
<td>445 ± 28</td>
</tr>
<tr>
<td>Celosian</td>
<td>25</td>
<td>7</td>
<td>203 ± 42.7*</td>
<td>83.2 ± 24**</td>
<td>92.9 ± 19**</td>
<td>255 ± 23**</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E., n = 7. **p < 0.01, *p < 0.05. Celosian and glycyrhrizin were administered intraperitoneally, three times, 24, 12 and 1 h before subcutaneous administration of CCL4. Blood samples were collected 24 h after CCL4 injection. N.D.: bilirubin level was less than 350 µg/dl.

Table 2. Effects of Celosian on CCL4-Induced Liver Injury by Histopathological Examination

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Necrosis</th>
<th>Fatty degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- + + + +</td>
<td>- + + + + + + + +</td>
</tr>
<tr>
<td>CCL4 treated control</td>
<td>0 0 3 4 0 0 5 2</td>
<td></td>
</tr>
<tr>
<td>Celosian (25mg/kg)</td>
<td>3 4 0 0 1 3 3 0</td>
<td></td>
</tr>
</tbody>
</table>

Intensity of the histopathological findings: observed by microscope and scored as (−) normal, (+) slight, (+++) moderate, (++++) severe.

Fig. 1. Photomicrograph of Liver 24 h after Carbon Tetrachloride Administration (H&E stained. × 100) Pretreated with Celosian

Rats were treated with 25 mg/kg of celosian at 24, 12 and 1 h before CCL4 administration. A) CCL4-treated control: massive hepatocellular necrosis surrounded by a rim of hydropic cells is seen. B) Celosian and CCL4-treated group; central vein is surrounded by a rim of hydropic cells, but no hepatocellular necrosis is seen. C) central vein. P, portal area.

Fig. 2. Effects of Celosian on α-GalN/LPS-Induced Liver Injury in Mice

Results are expressed as mean ± S.E., n = 10 or 11, **p < 0.01, *p < 0.05. Various doses of celosian were administered subcutaneously, 18 and 2 h before α-GalN/LPS intraperitoneal injection. Blood samples were collected 8 h after α-GalN/LPS administration.

Fig. 3. Notice the GPT level. In the control group, the GPT level hastily increased to 26841U/l after 8 h of administration of α-GalN/LPS, while the GPT levels in 1, 10 and 50 mg/kg of the celosian-treated group were 401, 135 and 84.5 U/l, respectively. The GPT level in 200 mg/kg of the glycyrhrizin treated group was 932 U/L. The results summarized in Fig. 2 indicate that celosian shows a dose-dependent protection against liver injury induced by LPS challenge to α-GalN sensitized mice.

Effects of Celosian on P. acnes/LPS-Induced Liver Injury in Mice In BALB/c mice i.v. injected with heat-treated P. acnes, a minimum amount of LPS was injected through the tail vein after 7 d of P. acnes treatment. More than 90% of the animals in the control group died within 24 h after LPS injection. On the other hand, the mortality of the celosian treated group was less than 30%. The results summarized in Fig. 3 indicate that celosian prevents liver injury induced by LPS challenge to P. acnes sensitized mice.

Effect of Celosian on LPO Generation in Vitro Celosian protected against liver damage in three types of liver injury models, hence, further investigation was carried out. At first, the effect of celosian on lipid peroxidation induced by ascorbic acid/Fe^{2+} in the presence of rat liver microsomes was tested. Celosian was incubated with rat liver microsomes in the presence of ascorbic acid and FeSO_4. After incubation, the amount of LPO was determined. Celosian at concentrations of 0.01, 0.1 and 1 mg/ml inhibited 22.6, 79.1 and 97.2% of LPO generation, re-
Fig. 3. Hepatoprotective Effects of Celosian on LPS-Induced Acute Hepatitis in Propionibacterium acnes Sensitized Mice
Six-week old male mice (12–13 per group) received heat-killed P. acnes (1 mg/mouse) and on day 7 they were given LPS (50 μg/mouse) by intravenous injection. Survival rate was observed. Celosian (○) or an equivalent amount of vehicle (●) was administered i.p. twice at a dose of 10 mg/kg, 18 and 2 h before LPS injection. Significant differences were expressed as *p<0.05 and **p<0.01.

Table 3. Effects of Celosian on Ascorbate/Fe^{2+}-Induced Lipid Peroxidation in the Presence of Rat Liver Microsomes

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (mg/ml)</th>
<th>n</th>
<th>MDA formation&lt;sup&gt;a&lt;/sup&gt; (nmol/ml)</th>
<th>Suppression&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>5</td>
<td>3.05 ± 0.156</td>
<td>—</td>
</tr>
<tr>
<td>Celosian</td>
<td>0.01</td>
<td>5</td>
<td>2.36 ± 0.285</td>
<td>22.6</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>4</td>
<td>0.64 ± 0.047&lt;sup&gt;**&lt;/sup&gt;</td>
<td>79.1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5</td>
<td>0.09 ± 0.073&lt;sup&gt;**&lt;/sup&gt;</td>
<td>97.2</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E., **p<0.01. The reaction mixture (1.0 ml) was composed of the microsomal suspension, ascorbate, FeSO<sub>4</sub>, and various concentrations of celosian. After incubation at 37°C for 20 min, lipid peroxide was measured by the method of Okihwa <i>et al.</i> a) Generation of lipid peroxide was calculated as the amount of malondialdehyde (MDA). b) Suppression (%) of MDA formation in reaction to the ascorbate/Fe<sup>2+</sup> treated control.

respectively, when compared to the control. The results shown in Table 3 clearly indicated that celosian remarkably inhibited the generation of LPO in a concentration dependent manner.

Effect of Celosian on in Vivo TNF-α Release in Mice Serum TNF-α concentration on d-GalN/LPS induced liver injury in mice was examined to elucidate the details of the hepatoprotective effect of celosian in immunological hepatitis. Blood samples in ddY mice were collected at 2 h prior, just before, and at 1, 2 and 4 h after d-GalN/LPS injection. Serum TNF-α (sTNF-α) level was measured by an ELISA kit, and the time course is shown in Fig. 4. Just before d-GalN/LPS injection, sTNF-α in the control group was almost zero, whereas sTNF-α was 1969 pg/ml in the celosian treated group. The sTNF-α level in the control group reached a peak at 1 h after d-GalN/LPS injection, and decreased to nearly zero after 4 h. The sTNF-α of the celosian treated group was found to be higher than that of the control. These findings indicate that celosian pretreatment not only induces sTNF-α but also enhances the sTNF-α area under the curve as compared with the control.

Effect of Celosian on TNF-α-Induced Liver Injury in Mice TNF-α/d-GalN-induced liver injury in mice was examined to elucidate immunologically the hepatoprotective effect of celosian. TNF-α i.v. injection 1 h after

Fig. 4. Effects of Celosian on TNF-α Release Following Administration of d-GalN and LPS in Mice Celosian (50 mg/kg, ○) or an equivalent amount of vehicle (●) was administered 18 and 2 h prior to d-GalN (700 mg/kg) and LPS (10 μg/kg) injection.

Table 4. Effects of Celosian on Recombinant Human TNF-α-Induced Liver Injury in d-GalN Sensitized Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose&lt;sup&gt;a&lt;/sup&gt; (mg/kg)</th>
<th>n</th>
<th>GPT level&lt;sup&gt;b&lt;/sup&gt; (IU/l)</th>
<th>HP effect&lt;sup&gt;c&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>—</td>
<td>10</td>
<td>44.5 ± 2.3</td>
<td>—</td>
</tr>
<tr>
<td>TNF-α treated</td>
<td>—</td>
<td>10</td>
<td>1174 ± 253</td>
<td>—</td>
</tr>
<tr>
<td>Celosian</td>
<td>50</td>
<td>11</td>
<td>75.9 ± 12.7&lt;sup&gt;**&lt;/sup&gt;</td>
<td>93.5</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E., significantly different from control, **p<0.01. Each mouse was injected i.p. with d-GalN (700 mg/kg) and after 1 h, recombinant human TNF-α (4 μg/kg) was administered i.v. a) Celosian or vehicle was subcutaneously administered 18 and 2 h before d-GalN injection. b) Blood sample was collected 8 h after d-GalN injection. c) Hepatoprotective effect (%) is due to GPT level against the TNF-α-treated control.

d-GalN sensitization caused liver damage in mice, and the blood GPT level in the control group reached 1174 IU/l at 8 h after d-GalN administration (7 h after TNF-α injection). On the other hand, the blood GPT level in the celosian treated group was 75.9 IU/l, which is almost the normal level, indicating no liver damage. The results summarized in Table 4 clearly suggest that celosian prevents TNF-α/d-GalN-induced liver injury in mice.

DISCUSSION

Celosian is an acidic polysaccharide, isolated as a hepatoprotective agent from the seeds of Celosia argentea. The results showed that celosian had liver protective action against CCl<sub>4</sub>-, d-GalN/LPS-, P. acnes/LPS- and d-GalN/TNF-α-induced liver injury animal models. This is the first report to find hepatoprotective activity of a polysaccharide and it is more effective than glycyrhrizin.

In CCl<sub>4</sub>-induced liver injury, CCl<sub>4</sub> is first metabolized to ·CCl<sub>3</sub> by such metabolic enzymes as cytochrome P450 in the hepatocellular microsome. This highly reactive radical injures the hepatocytes and its organelles by a direct physicochemical effect, that is, peroxidation of the membrane lipids, denaturation of proteins, or other chemical changes that lead to distortion or destruction. These changes comprise the first stage in the injury which culminates in necrosis and steatosis.2,24 Hepatoprotective agents such as glycyrhrizin and gomisin A in CCl<sub>4</sub>-induced
liver injury were reported to be due to their inhibitory effects on LPO generation.\textsuperscript{25,26} Hence, the effect of celoan on \textit{in vitro} lipid peroxidation was examined. LPO were non-enzymatically induced by ascorbate/Fe\textsuperscript{2+} in the presence of rat liver microsomes, and celosan inhibited LPO generation, though it is still unclear whether the hepatoprotective effect of celosan can be interpreted by the inhibition of LPO generation. As for the protection against d-Ga\textsuperscript{N}/LPS- and \textit{P. acnes}/LPS-induced liver injuries, other factors probably exist because of different mechanisms involved in the course of liver injury.

The mechanism of liver disorder on LPS and its related hepatitis is unclear, though a number of the pathophysiology of these immunologically mediated types of hepatitis have been investigated. The administration of endotoxin to animals can cause severe metabolic and physiological disturbances, leading to death. A common observation in such septic shock is acute liver failure. It has been established that macrophages (Kupffer cells) and their secretions, such as cytokines, superoxides, and nitric oxide (NO), mediate the action of LPS.\textsuperscript{27,28} Especially, TNF-\textgreek{z} is thought to be a terminal mediator and plays a main role in the cytotoxic effect.\textsuperscript{29} d-Ga\textsuperscript{N} is an inhibitor of protein biosynthesis through uridine trapping,\textsuperscript{29} and its effect is so specific in liver lesion that the sensitivity of hepatocytes to LPS highly increases because of the inhibition of acute phase protein induction.\textsuperscript{30–32} Therefore, the administration of both d-Ga\textsuperscript{N} and a very small amount of LPS can induce fulminating hepatitis through an immunological pathway. d-Ga\textsuperscript{N}/LPS-induced liver injury was also demonstrated to be mediated by TNF-\textgreek{z}.\textsuperscript{4,6,18} Moreover, the same can be applied to \textit{P. acnes}/LPS-induced liver injury. An initial injection of \textit{P. acnes} into mice and rats causes dense mononuclear cells infiltration into the liver. When the animals are then challenged with a normally innocuous amount of endotoxin, huge quantities of TNF-\textgreek{z} are produced from these infiltrating mononuclear cells, resulting in liver injury.\textsuperscript{31} TNF-\textgreek{z} has also been implicated in clinical liver diseases.\textsuperscript{12} Increases in serum TNF-\textgreek{z} levels have been found in patients suffering from fulminating hepatic failure, chronic hepatitis B virus infection and alcoholic hepatitis.\textsuperscript{33–35} Hence, pretreatment with down regulators of such TNF-\textgreek{z}-production as dexamethasone and prostaglandins or anti-TNF-\textgreek{z} antibodies not only can block experimental liver injuries induced by d-Ga\textsuperscript{N}/LPS or \textit{P. acnes}/LPS,\textsuperscript{17,36} but also have been clinically used to treat certain types of liver diseases.\textsuperscript{37–39}

In our investigation, celosan protected immunological liver injuries induced by d-Ga\textsuperscript{N}/LPS or \textit{P. acnes}/LPS. Moreover, we also investigated whether the hepatoprotective effects of celosan were localized in the suppression of TNF-\textgreek{z} release or the blocking of TNF-\textgreek{z} action. The effect of celosan on TNF-\textgreek{z} release was tested by measuring serum TNF-\textgreek{z} levels in d-Ga\textsuperscript{N}/LPS treated mice. The TNF-\textgreek{z} of the celosan treated group was higher than that of the control group. These findings indicate that celosan induces rather than inhibits the secretion of endogenous TNF-\textgreek{z} from macrophages. Some investigators have already reported that polysaccharides isolated from plants or crude drugs have an immunological activating property, including TNF-\textgreek{z} inducing activity.\textsuperscript{40,41} Celosan also appears to have such activities. In this model, the peak of endogenous TNF-\textgreek{z} appeared 1 h after the inducer administration. Therefore, exogenous TNF-\textgreek{z}, i.v. injected 1 h after d-Ga\textsuperscript{N} i.p. administration can induce the same type of hepatitis as that induced by d-Ga\textsuperscript{N}/LPS, and this model can be used for evaluation of the effects of celosan on the cytotoxic action of TNF-\textgreek{z} on hepatocytes. Celosan was able to protect against TNF-\textgreek{z}-induced hepatotoxicity in d-Ga\textsuperscript{N} sensitized mice. These observations indicate that the hepatoprotective effects of celosan on immunological liver injuries are probably due to blocking of the cytotoxic action of TNF-\textgreek{z} rather than the inhibition of TNF-\textgreek{z} release.

In conclusion, celosan, an acidic polysaccharide isolated from water extract of \textit{Celseia argentea}, showed a hepatoprotective effect against three different liver injury models induced by CCL\textsubscript{4}, d-Ga\textsuperscript{N}/LPS and \textit{P. acnes}/LPS. Moreover, celosan inhibited \textit{in vitro} LPO generation and also protected hepatocytes from injury induced by TNF-\textgreek{z}. We are still unable to predict the exact mechanisms of hepatoprotective effect of celosan; however, it is interesting to note that celosan inhibits LPO generation and blocks TNF-\textgreek{z} action, which have both been considered important parameters in hepatoprotective activity.

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