Effects of Dietary Corn Bran Hemicellulose and Neomycin on Hepatic Caspase-3 Activity and Glycoprotein Concentration in Rats Treated with or without D-Galactosamine

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Summary The effects of dietary corn bran hemicellulose (CBH) and neomycin (Neo) on hepatic caspase-3 activity and glycoprotein concentration were investigated to explore the possible mechanism of the alleviative action of dietary CBH and Neo on the development of D-galactosamine (GalN)-hepatitis. Rats were fed a diet containing 5% CBH with or without neomycin (Neo) for 7 or 14 d. On the last day of feeding, the rats were treated with GalN (400 mg/kg body weight, i.p.), and their plasma transaminase activities, hepatic glycoprotein concentrations and hepatic caspase-3 activities were determined 6 or 24 h later. Although the elevations of plasma transaminase activities were suppressed by CBH or Neo 24 h after GalN-treatment, the activities were not affected by CBH or Neo at an early stage (6 h) of GalN action. At 6 h, hepatic caspase-3 activity was elevated by CBH diet alone as high as that of the GalN-injected control-diet group, and the activity was not elevated further by GalN. At the same time, both GalN-treatment and CBH feeding reduced the hepatic glycoprotein (Mw . 64,000–74,000) concentration, but Neo did not affect the caspase activity or the glycoprotein concentration. These results suggest that dietary CBH elevates hepatic caspase-3 activity and reduces hepatic glycoprotein concentration, and may imply that CBH would suppress GalN-hepatitis not at the early- or middle-step of apoptosis but at the late-step of apoptosis or necrosis, although the relation between these phenomena and the alleviative effects of CBH and Neo on GalN-induced hepatitis is yet to be clarified.

Key Words D-galactosamine, glycoprotein, caspase-3 activity, rats, corn bran hemicellulose

Although extensive research has been conducted on the treatment of liver injury, sufficient studies on the prevention of the onset of hepatitis have not been performed. Lactulose is an oligosaccharide that is effective in mitigating the encephalopathy caused by severe hepatitis (1), and it has been demonstrated to reduce the elevation of plasma transaminase activities associated with the development of hepatitis caused by D-galactosamine (GalN), which causes acute hepatitis in experimental animals (2). We have also reported that some oligosaccharides containing D-galactose residue alleviated the elevations of plasma transaminase activities in rats injected with GalN (3), and our recent study showed a similar protective effect of corn bran hemicellulose (CBH) when given to Wistar strain rats. The results suggested that the GalN-dependent increase in plasma transaminase activities was suppressed by dietary CBH, and that the suppression is partly attributable to the increased level of glutathione in the liver (4).

However, the precise mechanism has not been elucidated. GalN-induced hepatitis has been well studied as a model of hepatitis that resembles viral hepatitis histologically (5). Some investigators have indicated that an increase in blood endotoxin concentration after injection of GalN causes hepatic injury associated with secretion of cytokines, such as TNF-α and IL-6 from Kupffer cells (6, 7). Endotoxin is a constituent of the cell wall of gram-negative bacteria, and since it may be absorbed from the gastrointestinal tract into the portal venous circulation, the intestinal microflora have generally been considered to be important for the development of GalN-hepatitis. The induction of GalN-hepatitis has recently been suggested to be initialized by apoptosis, which would be caused by cytotoxic cytokines released from Kupffer cells by the stimulation of endotoxin or bacteria translocated from the digestive tract (8, 9). On the other hand, GalN is thought to induce hepatotoxicity by inhibiting the synthesis of RNA and protein in the liver by decreasing intracellular UTP concentration, which finally leads to the necrosis of liver cells (5, 10–12). In addition, there are some reports in which the synthesis of hepatic glycoprotein, including
acute phase protein such as α1-antitrypsin and α1-acidglycoprotein, is suggested to be inhibited by GalN-metabolites, and this inhibition might be concerned with the hepatic injury from GalN (13). Some reports have described reduced glycoprotein synthesis in the liver of rats administered with GalN (14). From these contexts, GalN-hepatitis has been thought to be developed by the overlapping turbulence of the hepatocyte metabolism and immunological action, which might destroy the hepatocytes by apoptosis at the beginning, and the succeeding necrosis.

Corn bran hemicellulose is an indigestible polysaccharide that contains arabinoxylan, which is abundant in cereals and frequently consumed by humans in daily life. It is well known that dietary fiber is an important food component and that the substances in it have many physiological functions, such as normalization of plasma cholesterol levels (15), improvement of constipation, and protection from colon cancer (16, 17). These functions have generally been suggested to be mainly based on the fermentation of dietary fiber by intestinal bacteria, in addition to its own physicochemical properties.

To explore the possible mechanism of the alleviative action of dietary CBH on the development of GalN-hepatitis, we postulated that hepatic apoptosis, which is caused by cytotoxic cytokines derived from Kupffer cells activated by translocated endotoxin or bacteria, could be affected by dietary CBH or neomycin (Neo), and also that the hepatic glycoprotein synthesis might be influenced by the low molecular saccharides produced from dietary CBH by intestinal fermentation, which could be suppressed by an inabsorbable antibiotic Neo. Therefore, in the present study, the effects of dietary CBH and Neo on the activity of hepatic caspase-3, a biomarker of apoptosis, and on the concentration of hepatic soluble glycoproteins, were investigated in rats treated with or without GalN. In these studies we examined the enzyme activity and glycoprotein concentration mainly at the early stage of the development of GalN-hepatitis to avoid the secondary effect of the necrosis of hepatocytes that would occur at a later stage.

MATERIALS AND METHODS

Materials. The following materials were obtained commercially: D-galactosamine hydrochloride, Transaminase C-II-test (a transaminase assay kit) from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); sodium pentobarbital (Nembutal) solution from Dynabot (Osaka, Japan); ECL glycoprotein detection system and ECL Western blotting analysis system from Amersham Biosciences K.K. (Tokyo, Japan). Corn bran water-soluble hemicellulose (CBH), which is commercially available as Cell-ace, was a kind gift from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). The dietary fiber content of the CBH preparation was 86%, and composed of 45.7% of L-arabinose and 31.5% of L-xylose and other saccharide residues. Other reagents were purchased from Wako Pure Chemical Industries, Ltd.

Animals and feeding. For all the experiments, four-week-old male Wistar rats (Clean) were obtained from Japan SLC (Hamamatsu, Japan). These rats were about two-fold sensitive to GalN as compared with the same strain obtained from CLEA Japan, Inc. (Tokyo, Japan) or Charles River Japan (Yokohama, Japan) in our preliminary experiments. The rats were kept in an environmentally controlled room at a temperature of 22±1°C with a 12 h light (7:00–19:00) and dark cycle. The experimental diets were prepared based on the composition of AIN-76™ as shown in Table 1. When necessary, neomycin (Neo, Wako Pure Chemical Industries, Ltd.) was mixed in the diet. Three separate experiments were conducted in this study. All rats were fed a commercial diet (CE-2, CLEA Japan, Inc.) for 3 d, then a control (Cont) diet for 4 d, after which the rats were divided into several groups of 5–8 animals each and fed the experimental diets. In all cases, the rats were allowed free access to their respective experimental diets and drinking water except for the time of GalN injection, as described below. The rats in GalN-administered groups were injected with GalN (400 mg/kg body weight, intraperitoneally) after being fed experimental diets. The aqueous D-galactosamine solution used for injection was prepared by adjusting its pH to 7.0 and its concentration to 300 mg/mL, and it was sterilized by membrane filtration before use. In all experiments the Cont and CBH diet groups injected and not injected with GalN are referred to as the Cont(+GalN) group, CBH+GalN) group, Cont(−GalN) group, and CBH(−GalN) group, respectively. In the experiment in which Neo was placed in the Cont or CBH diet the groups are referred to as Cont+Neo or CBH+Neo, and when these groups were injected with GalN, they are referred to as Cont+Neo(+GalN) and CBH+Neo(+GalN) groups, respectively.

In experiment 1, four groups of 8 rats each were fed the Cont, CBH, Cont+Neo, or CBH+Neo diet for 14 d. Diets were withheld for 4 h before and after the GalN-injection (8 h in total). Twenty-four hours after injection, blood was drawn from the posterior vena cava into

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**Table 1. Composition of the experimental diet (%).**

<table>
<thead>
<tr>
<th></th>
<th>Cont</th>
<th>Cont+Neo</th>
<th>CBH</th>
<th>CBH+Neo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Corn bran hemicellulose</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>40</td>
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<td>35</td>
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<tr>
<td>Sucrose</td>
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<td>25</td>
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<td>25</td>
</tr>
<tr>
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<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn oil</td>
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<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mixture¹</td>
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<td>3.5</td>
<td>3.5</td>
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<tr>
<td>Cellulose</td>
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<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Choline bitartrate</td>
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<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Neomycin</td>
<td>—</td>
<td>0.67</td>
<td>—</td>
<td>0.67</td>
</tr>
</tbody>
</table>

¹ Composition of the AIN-76™ diet.

Cont, Control diet; Cont+Neo, Control diet containing neomycin; CBH, CBH diet; CBH+Neo, CBH diet containing neomycin.
In experiment 2, the caspase-3 activity and glycoprotein concentration in the liver of the rats were measured 6 h after GalN injection, a comparatively early stage in the development of GalN-hepatitis. One half of 4 groups of 6 rats each were fed with the Cont diet and others with the CBH diet for 7 d. Thereafter one group in each diet group was injected with GalN solution in the same manner as in experiment 1, and the other groups were injected with the same volume of saline at the end of the feeding period. Their diets were removed 4 h before GalN injection, and they were fasted thereafter. Six hours after GalN injection, dissection and blood collection were performed as described in experiment 1. The liver was quickly excised, immediately frozen in liquid nitrogen, and stored at −80°C, until used to assay caspase-3 activity and measure the glycoprotein concentration.

In experiment 3, the effects of dietary CBH with or without Neo on the hepatic caspase-3 activity and glycoprotein concentration were examined 6 h after GalN injection. Twenty-nine rats were fed the Cont, CBH, Cont+Neo or CBH+Neo diet for 7 d, and at the end of the feeding period all rats except the Cont (−GalN) group were injected with GalN solution in the same manner as in experiment 1, and the rats in the Cont (−GalN) group were injected with the same volume of saline. Their diets were removed 4 h before GalN injection, and they were fasted thereafter. Six hours after GalN injection, dissection and blood collection were performed as described in experiment 1, and the liver was treated and preserved as described in experiment 2. The care and treatment of the rats were carried out according to the guidelines prescribed in the Faculty of Horticulture of Chiba University, and National Institutes of Health Guide for the care and use of laboratory animals (19).

Biochemical Analyses. The activities of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed with commercial assay kits (Transaminase C-II-test, Wako Pure Chemical Industries, Ltd.). The liver was homogenized with 9 volumes of 0.25 M sucrose solution at 0°C, and the supernatant was collected after centrifugation at 105,000×g for 60 min at 4°C. The protein concentration was adjusted to 200 μg/mL, and the protein (15 μL) was electrophoresed according to the methods described by Laemmli et al. (20). The protein bands were then transferred to a PVDF membrane. The bands of transferrred glycoprotein were detected with an ECL glycoprotein detection kit (Amersham Biosciences K.K.) and ECL Western blotting analysis system that can detect all kinds of saccharides after it metaperiodate oxidation and derivatization to biotin-hydrazones using streptavidin-HRP conjugate, according to the manufacturer’s instruction. The relative concentrations of the glycoprotein bands were analyzed with an image analyzer LAS-1000 (Fuji Film, Japan).

For caspase-3 assay, the liver was homogenized with 9 volumes of physiological saline at 0°C, and after centrifugation at 105,000×g for 60 min at 4°C, the supernatant was collected. The caspase-3 activity in the cytoplasm was analyzed with a CPP32/caspase-3 fluoromeric protease assay kit (Medical & Biological Laboratories, Japan) according to Murata et al. (21). In brief, the activity was assayed by measuring the fluorescence of 7-amino-4-trifluoromethyl coumarin (excitation: 400 nm, emission: 505 nm) liberated from fluorogenic substrate DEVD-7-amino-4-trifluoromethyl coumarin by caspase-3 during 1 h of incubation at 37°C. It was confirmed that the effect of non-specific proteases in the supernatant was negligible because of the use of a protease inhibitor PMSF (1 mM).

Statistical analysis. All values obtained in experiment 1 were evaluated by two-way ANOVA and partly by Student’s t-test. In experiments 2 and 3, the statistical evaluation was performed by the Bonferroni test, and partly by Student’s t-test after the analysis by two-way ANOVA (The Statistical Program for the Social Sciences (SPSS) version 11.0, SPSS Inc.). The level of significance was p<0.05.

RESULTS
Effect of dietary neomycin and/or CBH on the induction of GalN-hepatitis 24 h after GalN-treatment (Exp. 1).

The body weight gain, feed efficiency, carcass weight, liver weight, liver ratio ([Liver weight/Carcass weight]×100), and plasma transaminase (AST and ALT) activities in experiment 1 are shown in Table 2. Body weight gain was not significantly affected by CBH and Neo, but feed efficiency was slightly increased by Neo. The plasma ALT and AST activities in the Cont+Neo (−GalN) and CBH+Neo (−GalN) groups were significantly lower than those in the Cont (−GalN) and CBH (−GalN) groups. When analyzed by two-way ANOVA, CBH and Neo were shown to have depressed the plasma ALT and AST activities in the GalN-treated groups.

Influence of CBH diet on hepatic caspase-3 activity and glycoprotein concentration 6 h after GalN injection (Exp. 2).

The results of experiment 2 are shown in Fig 1. Hepatic caspase-3 activities in the Cont (−GalN) and CBH (−GalN) and CBH+GalN) groups were found to be significantly higher than those in the Cont (−GalN) group, although the results of two-way ANOVA indicated that the CBH diet and GalN-treatment did not affect hepatic caspase-3 activity. A part of the results of experiment 2 including plasma ALT and AST activities were described in another report (4). In brief, body weight gain was unaffected by CBH diet or GalN, and liver weight and its ratio against carcass weight were reduced or increased by the ingestion of the CBH diet or GalN-treatment, respectively. In addition, the cecum weight and its ratio to carcass weight were approximately doubled by the CBH diet. The plasma transaminase activities of the groups in experiment 2 were as follows (ALT
Fig. 1. The effect of CBH diet on hepatic caspase-3 activity 6 h after GalN treatment (Exp.2). All values are means±SEM (n=6). Values of bars not sharing common superscript letters are significantly different (p<0.05) when analyzed by the Bonferroni test. The statistical significance of the effects was also analyzed by two-way ANOVA, using diet and GalN as factors. The results are indicated in the text.

and AST, U/L): Cont(−GalN) [5.75±0.36, 30.51±0.86], Cont(+GalN) [38.51±6.40, 94.3±9.32], CBH (−GalN) [8.13±1.20, 36.4±3±1.04], CBH(+GalN) [32.96±12.08, 82.3±22.4] (4). Plasma AST and ALT activities at 6 h after GalN-injection were significantly elevated as were those in experiment 1, but the elevation of the enzyme activities were not suppressed by the CBH diet at the early stage of hepatic injury.

These results indicated that hepatic caspase-3 activity was induced by the CBH diet alone and that the induction of this enzyme by GalN in the rats fed the Cont diet was not observed in the rats fed the CBH diet. Figure 2 shows that the concentration of hepatic glycoprotein (Mw 64,000–74,000) was significantly lower in the Cont(+GalN) group than in the Cont(−GalN) group, and that the ingestion of CBH without GalN (CBH(−GalN) group) was found to result in a low glycoprotein concentration (by Bonferroni test), although the results by ANOVA demonstrated that the CBH diet and GalN did not affect the glycoprotein concentration. Influence of CBH diet and Neo on hepatic caspase-3 activity and glycoprotein concentration 6 h after GalN injection (Exp. 3).

The body weight gain, feed efficiency, carcass weight, liver weight, and liver ratio in experiment 3 are shown in Table 3. The results of two-way ANOVA (factors: diet and Neo) for the 4 groups with GalN-treatment indicated that CBH and Neo significantly affected feed efficiency, cecum weight and the ratio of cecum weight against carcass weight, and that CBH was also effective in the weight gain. Feed efficiency was significantly higher in the CBH+Neo(+GalN) group than in the other groups. The cecum weight and its ratio against carcass weight were significantly higher in the groups ingesting CBH with or without Neo than in the Cont groups, irrespective of GalN-treatment. The activity of plasma ALT activity was significantly lower in the Cont(−GalN) group than in other groups. The AST activity in the Cont(−GalN) group was significantly
lower than that in the CBH+Neo(+GalN) group.

Figure 3 shows that caspase-3 activity in the Cont(+GalN) group was significantly higher than that in the Cont(−GalN) group. Such induction of caspase-3 activity by GalN was not significantly depressed by Neo, and the activities in the CBH(+GalN) groups with and without Neo were slightly, but not significantly, lower than that in the Cont(+GalN) group (by Bonferroni test). The effects of neomycin and CBH on the relative concentration of a soluble glycoprotein with a molecular weight of 64,000–74,000 are shown in Fig. 4. The glycoprotein concentration was significantly lower in all GalN-treated groups than in the Cont(−GalN) group. The groups ingesting Neo, (Cont+Neo(+GalN) and CBH+Neo(+GalN) groups), or the group ingesting CBH, (CBH(+GalN) group), demonstrated slightly higher glycoprotein concentrations than in the Cont(+GalN) group, but the differences between the values were not significant (by Bonferroni test).

DISCUSSION

This study was performed to make clear the effect of CBH and Neo intake on hepatic caspase-3 activity and glycoprotein concentration as a part of the research for clarifying the alleviative mechanism of CBH on GalN-induced hepatic disorder. Neomycin was used in experiments 1 and 3 to investigate the involvement of intestinal microflora in the suppressive action of CBH. A CBH feeding-period of 14 d was planned in experiment 1, but because the suppressive effect of CBH was observed by 7 d (4), a feeding period of 7 d was used in experiments 2 and 3. Since the previous study also indicated that plasma transaminase activities began to rise around 6 h after GalN-treatment (22), caspase-3 activities and the glycoprotein concentrations in experiments 2 and 3 were measured 6 h after GalN administration, a relatively early stage in the development of GalN-hepatitis.

The results of experiment 1 indicated that the elevation of plasma ALT and AST activities 24 h after GalN-treatment was significantly suppressed by Neo as well as by dietary CBH. A protective effect of Neo against GalN-hepatitis has also been reported by Katayama et al. (23). Iwaki et al. (24) found that hepatic injury and endotoxemia were not observed when GalN was administered to cecrectomized rats, and considered that the effect of intestinal bacteria and endotoxin must be involved in GalN-hepatitis. The suppressive action of Neo on GalN-induced hepatic injury was therefore deduced to be attributable to the reduction of intestinal bacteria and endotoxin. The suppressive effect of Neo on the plasma transaminase activity (reduced to around 18% in ALT and 15% in AST activities) was greater than that of CBH (reduced to around 27% in ALT and 32% in AST activities), and CBH in the presence of Neo (CBH+Neo(+GalN) group) suppressed ALT activity to around 74% and AST activity to 93% of those of the Cont(−GalN) group (Table 2). A significant difference in the plasma AST or ALT activity was not shown between the Cont+Neo(+GalN) and CBH+Neo(+GalN) groups. Because of the suppressive effect of Neo itself, it would be difficult to determine whether intestinal bacteria were necessary for the effect of CBH. The appearance of the interactive effect of the diets and Neo (diets×Neo) might indicate that intestinal bacteria were involved in the suppressive action of CBH on the elevation of these enzyme activities caused by GalN (Table 2).

Although the plasma activities of AST and ALT in experiments 2 and 3 at 6 h after the GalN injection were lower than those at 24 h (experiment 1), the activities of both enzymes were significantly elevated or
Table 3. Effect of neomycin on the body weight gain, feed efficiency, carcass weight, liver weight, cecum weight, and plasma transaminase activities in the experimental animals (Exp. 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cont (−GalN)</th>
<th>Cont (+GalN)</th>
<th>Cont + Neo (+GalN)</th>
<th>CBH (+GalN)</th>
<th>CBH + Neo (+GalN)</th>
<th>ANOVA</th>
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<tbody>
<tr>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Neomycin</td>
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<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>Diet</td>
</tr>
<tr>
<td>GalN</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Neo</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Diet×Neo</td>
</tr>
<tr>
<td>Weight gain (g/7d)</td>
<td>21.4±1.72</td>
<td>22.66±0.76</td>
<td>19.66±1.11</td>
<td>22.16±0.90</td>
<td>23.81±0.54</td>
<td>p&lt;0.05</td>
</tr>
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<td>Feed efficiency1(%)</td>
<td>30.17±1.4a</td>
<td>32.53±0.62a</td>
<td>32.87±1.18a</td>
<td>33.23±0.64a</td>
<td>37.61±0.62b</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Carcass weight (g)</td>
<td>96.6±4.61</td>
<td>94.33±1.74</td>
<td>89.33±1.92</td>
<td>93.5±2.74</td>
<td>89.83±2.35</td>
<td>NS</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>5.17±0.24</td>
<td>5.61±0.17</td>
<td>5.02±0.31</td>
<td>5.37±0.2</td>
<td>5.29±0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Ratio2(%)</td>
<td>5.36±0.22</td>
<td>5.96±0.25</td>
<td>5.61±0.27</td>
<td>5.77±0.29</td>
<td>5.89±0.22</td>
<td>NS</td>
</tr>
<tr>
<td>Cecum weight (g)</td>
<td>2.03±0.27a</td>
<td>1.96±0.12a</td>
<td>5.46±0.31b</td>
<td>3.98±0.18b</td>
<td>9.47±0.37b</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Ratio3 (%)</td>
<td>2.1±0.24a</td>
<td>2.08±0.11a</td>
<td>6.1±0.28b</td>
<td>4.26±0.20b</td>
<td>10.58±0.52d</td>
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<td>Plasma transaminase activities</td>
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<tr>
<td>ALT (U/L)</td>
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<tr>
<td>AST (U/L)</td>
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<td>78.26±17.32a</td>
<td>86.22±16.85a</td>
<td>83.56±12.74a</td>
<td>109.27±4.51b</td>
<td>NS</td>
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</table>

Cont, control diet; CBH, corn bran hemicellulose diet; GalN, galactosamine; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Values are expressed as mean±SEM. n=5 for Cont (−GalN) group and n=6 for other groups.

1 Body weight gain/Feed intake×100.

2 (Liver weight/Carcass weight)×100.

3 (Cecum weight/Carcass weight)×100.

ANOVA was adopted for 4 GalN-treated groups: Cont (+GalN), Cont + Neo (+GalN), CBH (+GalN) and CBH + Neo (+GalN). The statistical significance of difference among values was analyzed by the Bonferroni test. Values within each row not sharing common superscript letters are significantly different (p<0.05).
tended to be increased by GalN. Neither CBH nor Neo, however, alleviated the elevation of these enzyme activities by GalN at this stage (Table 3). Caspase-3 activity in the liver was observed to be significantly elevated 6 h after GalN administration (Figs. 1 and 3), and a similar induction of caspase-3 activity in the liver by GalN has been described by Jaspreet et al. (25). Hepatic caspase-3 activity was significantly higher in the CBH group without GalN (CBH(−GalN) group) than that of the Cont (−GalN) group, and its activity in the CBH group was not further elevated by GalN (Fig. 1). Since we were unable to find any other reports describing the effect of dietary fiber on hepatic caspase-3 activity, this may be the first report to describe elevation of hepatic caspase-3 activity by CBH. Although the hepatic caspase-3 activity was elevated by GalN, neither CBH nor Neo suppressed the elevation (Figs. 1 and 3). These results suggest that dietary CBH and Neo are unable to suppress apoptosis in the early stage of the induction of GalN-hepatitis. Although apoptosis has generally been thought to be involved in the development of GalN-hepatitis (26), the results of experiment 2 showed that the plasma AST and ALT activities in the CBH(−GalN) group were almost the same as those in the Cont (−GalN) group (4), despite the elevation of caspase-3 activity in CBH ingestion (Fig. 1). Recently we observed that dietary CBH increased liver GSH concentration. Therefore, CBH might suppress GalN hepatitis by inhib-

Fig. 3. The effect of dietary CBH and Neomycine on hepatic caspase-3 activity 6 h after GalN-treatment (Exp.3). All values are mean±SEM. n=5 for Cont(−GalN) group, and n=6 for other groups. Values of bars not sharing common superscript letters are significantly different (p<0.05) when analyzed by the Bonferroni test. The statistical significance of the effects was also analyzed by two-way ANOVA, using diet and Neo as factors. ANOVA was adopted for 4 GalN-treated groups: Cont(+GalN), Cont+Neo(+GalN), CBH(+GalN) and CBH+Neo(+GalN). The results are indicated in the text.

A

![Image of Fig. 3](image1)

B

<table>
<thead>
<tr>
<th>Group</th>
<th>Cont(−GalN)</th>
<th>Cont(+GalN)</th>
<th>Cont+Neo(+GalN)</th>
<th>CBH(+GalN)</th>
<th>CBH+Neo(+GalN)</th>
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<td>CBH</td>
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<td>Neomycine</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>GalN</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glycoprotein</td>
<td>64,000−74,000</td>
<td>40.70±4.67a</td>
<td>62.19±11.54a</td>
<td>51.53±5.75b</td>
<td>59.66±3.61b</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of dietary neomycin on the relative concentration of hepatic soluble glycoproteins with molecular weight 64,000–74,000 in rats 6 h after GalN-treatment (Exp.3). A: Representative patterns of duplicate samples in each group. B: Relative concentration of the sum of all bands within the molecular range between 64,000 and 74,000. The value of Cont(−GalN) was expressed as 100%. All values are mean±SEM. n=5 for Cont(−GalN) group, and n=6 for other groups. Values not sharing common superscript letters are significantly different (p<0.05) when analyzed by the Bonferroni test. The statistical significance of the effects was also analyzed by two-way ANOVA, using diet and Neo as factors. ANOVA was adopted for 4 GalN-treated groups: Cont(+GalN), Cont+Neo(+GalN), CBH(+GalN) and CBH+Neo(+GalN). The results are indicated in the text.
iting oxidative stress at the late step of apoptosis or necrosis. Because TNF-α was suggested to mediate the development of GalN-hepatitis, and Wallach et al. (27) reported that preceding administration of TNF-α caused desensitization of mice to a lethal amount of TNF-α, it would be likely that CBH desensitized the rats against GalN in the present study.

The concentration of UDP-galactosamine in the liver of the rats increased rapidly after the injection of GalN (7–10), and since the increase was accompanied by a reduction in the concentration of UTP and UDP-glucose in the liver, it is likely that the hepatotoxic action of GalN is mediated by suppression of RNA or glycoprotein biosynthesis caused by the deficiency of UTP or related compounds. Bauer et al. (28) have reported inhibition of overall protein and glycoprotein secretion by GalN, and that was partially explained by inhibition of de novo protein synthesis. Decreased plasma protein bound to carbohydrates and decreased plasma concentration of the glycoprotein α1-antitrypsin after GalN-treatment have been found by Bolmer and Kleinerman (29), but no detailed analysis of the effects of GalN on the glycosylation of hepatitis-specific proteins has ever been published. The results of experiment 2 suggested that administration of GalN significantly reduced the hepatic glycoprotein concentration in the Cont diet group. This result was similar to that shown by Bauer et al. (28), who speculated that normal glycosylation of cell membrane structural glycoproteins might be impaired by GalN and that the resulting alterations in the glycoprotein pattern caused the liver cell injury after GalN administration. Ingestion of CBH in the present experiments, also caused the reduction of the concentrations of some glycoproteins in the liver even in the absence of GalN-treatment (Fig. 2).

The glycoprotein concentration in the CBH group was not decreased further by GalN. Still, the reduction in hepatic glycoprotein concentration at 6 h after the GalN injection and elevation of plasma ALT and AST activities at 24 h may suggest that the development of GalN-hepatitis is related to the deficiency of some hepatic glycoprotein. On the other hand, Neo did not significantly affect the decrease in glycoprotein concentration induced by GalN, although the effect of Neo in the absence of GalN was not determined (Fig. 4). In the present study we selected glycoproteins having molecular weights between 64,000 and 74,000 as representatives of the glycoprotein of those affected by GalN because their concentration was greatly decreased by GalN and the decrease was easily observable. However, representative acute phase proteins, α1-antitrypsin and α1-acidglycoprotein are not included in these proteins. So, further investigation will be necessary to determine the glycoprotein that is critically important to the development of GalN-hepatitis.

From these results, neither CBH nor Neo was considered to affect the elevation of plasma AST and ALT activities at 6 h after GalN-injection, although the increase in their activities at 24 h after the GalN-injection was suppressed by both treatments. Because dietary CBH alone increased hepatic caspase-3 activity and decreased the glycoprotein concentration in the liver without altering the plasma transaminase levels, caspase-3 and glycoprotein were not considered to be directly correlated with the elevation of transaminase level in the early stage of GalN-hepatitis, although they were affected by the GalN-injection. The suppressive effect of dietary CBH on the elevation of transaminase levels at a later stage of GalN-hepatitis (24 h after the injection), however, may be related to caspase-3 and glycoprotein.

In conclusion, dietary CBH was demonstrated to cause the elevation of hepatic caspase-3 activity and the reduction of hepatic glycoprotein (Mw. 64,000–74,000) concentration in the absence of GalN. In addition, in the early stage of GalN hepatitis (6 h after GalN treatment), the caspase-3 activity was elevated by the CBH diet as high as that of the GalN-injected control diet group, and the activity in the CBH diet group was not elevated further by GalN. The hepatic glycoprotein concentration was reduced by both GalN-treatment and CBH feeding, but Neo did not affect them at the same stage. These results may imply that CBH would suppress the GalN-hepatitis not at the early- or middle-step of apoptosis but at the late-step of apoptosis or necrosis. However, the relation between these phenomena and the alleviative action of CBH on GalN-induced hepatitis is still unclear. Further study is necessary to elucidate the mechanism of the alleviative action of dietary CBH on GalN-hepatitis.

REFERENCES


