Short Communication

Antioxidative and hepatoprotective effects of fructo-oligosaccharide in d-galactose-treated Balb/cJ mice

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Abstract
Chronic subcutaneous (s.c.) administration of d-galactose (DG) to BALB/cJ mice has been shown to induce oxidative stress and is considered a model to mimic accelerated ageing. Fructo-oligosaccharide (FO) is a well-defined prebiotic and its fermentation by lactic acid bacteria has been shown to exert antioxidative capacity. The present study was aimed to determine whether FO attenuated DG-induced oxidative stress and hepatopathy in Balb/cJ mice. Mice (12 weeks of age, n = 40) were divided into control (s.c. saline), DG (s.c. 1.2 g/kg body weight), DG + FO (5%, w/w) and DG + vitamin E (0.2%, w/w) groups and were killed after 52 d of treatment. Results indicated that DG significantly decreased the hepatic superoxide dismutase and glutathione peroxidase activities. These alterations were ameliorated both by FO and vitamin E. DG increased the hepatic TAG content approximately by 7.2% compared with the vehicle control, which was in agreement with the histological alteration. FO, similar to vitamin E, almost normalised the hepatic TAG content and ameliorated the histological characteristics of fatty liver. Similarly, the increased plasma alanine aminotransferase activity induced by DG was normalised by FO and vitamin E, respectively. Faecal bifidobacteria counts were greater in the DG + FO and DG + vitamin E groups compared with the DG group, respectively. In conclusion, the present study indicated that FO diminished the altered hepatic antioxidative enzyme activities and morphology caused by chronic DG administration in Balb/cJ mice, partially associated with its prebiotic role in the colon.

Key words: d-Galactose; Fructo-oligosaccharide; Antioxidative capacity; Liver; Ageing

One of the well-recognised ageing theories indicates that generation of oxidative stress leads to cell and tissue damage and ultimately results in ageing and cell death(1). Chronic administration of d-galactose (DG) to BALB/cJ mice has been shown to induce changes which resemble accelerated ageing, such as formation of advanced glycation end products(2,3), neurological impairment(4,5), decreased serum antioxidative enzyme activities(4,5) and inflammation in the liver(5,6). Several antioxidants(3,7,8) and Chinese herbs(9) have been shown to attenuate the ageing damage in C57BL/6J mice treated with DG.

Fructo-oligosaccharide (FO), a well-defined prebiotic(10), has been incorporated into drinks and desserts to improve bowel function in elderly persons(11). Recent clinical studies have initially shown that the consumption of a prebiotic mix(12) and a FO supplement(13) beneficially reduces the blood indices of peroxidation status. However, the effect of FO in DG-induced hepatic oxidative damage has never been demonstrated.

The main goal of the present study was to assess the anti-ageing and hepatoprotective effects of FO in DG-administered Balb/cJ mice, by the determination of antioxidative enzyme activities and the morphology of the liver and the biochemical indices of liver function.

Materials and methods

Animals and diets

Male Balb/cJ mice (10 weeks old) were obtained from the BioLASCO Taiwan Company Limited (Taipei, Taiwan,

Abbreviations: DG, d-galactose; FO, fructo-oligosaccharide.

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British Journal of Nutrition

Normal saline (control (subcutaneous saline, basal diet), DG (subcutaneous 1:2 g D-galactose/kg body weight, basal diet), DG + FO (50 g active ingredients/kg basal diet) and DG + vitamin E (2 g α-tocopherol/kg basal diet, as an antioxidant positive control) and were killed after 52 d of treatment. The basal diet consisted of a ground rodent chow (Lab 5001; Purina Mills, St Louis, MO, USA) and sucrose that match the digestible sugar present in the FO syrup (Institute of Microbial Resources, Taichung, Taiwan, ROC) (11). The mixed powder diet was then re-formed into a small dough with deionised water in order to balance the liquid content among diets and to reduce spillage. The doses of supplements were used based on previous studies, indicating that supplementing 5% (w/w) FO into a fibre-free diet beneficially modulated colon microflora and reduced faecal toxicity (14), while 0.2% (w/w) α-tocopherol altered pro-oxidation status in rats (15). All animals were allowed to have free access to water and food during the study. Animal care followed the guidelines of the National Research Council (16) and was approved by the Institutional Animal Care and Use Committee in the Chung Shan Medical University. The mice were placed in metabolism cages during days 44–49, while fresh faeces were collected and frozen within 30 min of excretion. Faecal samples were lyophilised and kept at −20°C for further analyses of microflora. Microbial DNA was isolated and amplified using PCR. A portion of each liver was homogenised and extracted with 10 vol (v/w) of 0.1 M phosphate buffer (pH 7.4, containing 1 mM-EDTA). A portion of each liver was homogenised in 10 vol (v/w) of 0.1 M phosphate buffer (pH 7.4, containing 1 mM-EDTA). A portion of each liver was homogenised and extracted with 10 vol (v/w) of 0.1 M phosphate buffer (pH 7.4, containing 1 mM-EDTA). A portion of each liver was homogenised and extracted with 10 vol (v/w) of 0.1 M phosphate buffer (pH 7.4, containing 1 mM-EDTA).

Histological routine. Paraffin sections (4 μm) were used to quantify bifidobacteria and total bacteria, respectively (23). Probe fluorescence was detected with a Zeiss Axioskop2 microscope (Carl Zeiss, Jena, Germany), as described previously (24).

Histological evaluation

Liver tissues were fixed in Bouin’s solution overnight and then processed for histological routine. Paraffin sections (4 μm) were mounted on microscope slices and stained with haematoxylin and eosin. Histological evaluation was done under 200× magnification.

Statistical analyses

Data were presented as means with their standard errors and analysed using SPSS version 14 (SPSS, Inc., Chicago, IL, USA). Parametric data and log-transformed bacteria counts were analysed by one-way ANOVA, followed by the least significant difference test. Differences were considered significant at P<0.05.

Results

Body and liver weights

There were no significant differences in body weight and weight gain among groups (data not shown). Relative liver weight (percentage of body weight) was similar among groups, 4.7 (SE 0.6), 4.7 (SE 0.5), 4.6 (SE 0.4) and 4.7 (SE 0.6)% for the control, DG, DG + FO and DG + vitamin E groups, respectively.
Antioxidative enzyme activities and TAG content in the liver

Superoxide dismutase and glutathione peroxidase activities were significantly suppressed by DG treatment by approximately 32.3% (P<0.001 vs. control) and approximately 29.7% (P=0.016 vs. control), respectively (Table 1). Both FO (P<0.001 vs. DG) and vitamin E (P<0.001 vs. DG) reversed the DG-induced decrease in superoxide dismutase activity, and tended to ameliorate (P=0.49 for DG+FO vs. control; P=0.11 for DG+vitamin E vs. control) the DG-induced decrease in glutathione peroxidase activity. Catalase activity was non-significantly altered by DG (P>0.05 vs. control). However, catalase activity in the DG+vitamin E group was raised by approximately 20.0% (P=0.049 vs. DG). Hepatic TAG concentration was 234.4 (SE 5.6), 238.6 (SE 3.4) and 243.8 (SE 5.6) μmol/g liver in the control, DG+FO and DG+vitamin E groups, respectively, all of which were significantly lower than that shown in the DG group, 252.6 (SE 3.4) μmol/g liver (P<0.05, respectively).

Table 1. Hepatic antioxidative enzyme activities in d-galactose-treated Balb/cJ mice
(Mean values with their standard errors, n=6)

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (IU/mg protein)</th>
<th>GPx (IU/mg protein)</th>
<th>Catalase (IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80.0 ± 5.7</td>
<td>46.2 ± 4.2</td>
<td>844.7 ± 53.9</td>
</tr>
<tr>
<td>DG</td>
<td>68.1 ± 4.9</td>
<td>32.5 ± 3.3</td>
<td>726.2 ± 49.5</td>
</tr>
<tr>
<td>DG + FO</td>
<td>78.4 ± 3.4</td>
<td>41.1 ± 2.0</td>
<td>793.6 ± 41.9</td>
</tr>
<tr>
<td>DG + vitamin E</td>
<td>82.4 ± 3.5</td>
<td>37.5 ± 4.5</td>
<td>871.2 ± 49.8</td>
</tr>
</tbody>
</table>

SOD, superoxide dismutase; GPx, glutathione peroxidase; DG, d-galactose; FO, fructo-oligosaccharide.

Antioxidative enzyme activities and TAG content in the liver

Table 1 shows the mean values with their standard errors for SOD, GPx, and Catalase activities in the liver of Balb/cJ mice treated with d-galactose (DG) and various treatments. The values are presented as mean ± standard error (n=6). The study revealed that superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were significantly suppressed by DG treatment by approximately 32.3% (P<0.001 vs. control) and approximately 29.7% (P=0.016 vs. control), respectively. Both FO (P<0.001 vs. DG) and vitamin E (P<0.001 vs. DG) reversed the DG-induced decrease in superoxide dismutase activity, and tended to ameliorate (P=0.49 for DG+FO vs. control; P=0.11 for DG+vitamin E vs. control) the DG-induced decrease in glutathione peroxidase activity. Catalase activity was non-significantly altered by DG (P>0.05 vs. control). However, catalase activity in the DG+vitamin E group was raised by approximately 20.0% (P=0.049 vs. DG). Hepatic TAG concentration was 234.4 (SE 5.6), 238.6 (SE 3.4) and 243.8 (SE 5.6) μmol/g liver in the control, DG+FO and DG+vitamin E groups, respectively, all of which were significantly lower than that shown in the DG group, 252.6 (SE 3.4) μmol/g liver (P<0.05, respectively).

Histopathological observation

The liver histological study was conducted to determine the protective effect of FO on DG-induced injury. The hepatocytes in the DG group were filled with lipids in the absence of the nucleus (Fig. 1(b)). However, this histological alteration was not observed in the control (Fig. 1(a)) group, and was ameliorated in the presence of FO (Fig. 1(c)) and vitamin E (Fig. 1(d)).

Faecal microflora

The faecal bifidobacteria concentration was the lowest in the DG group, which was significantly increased by FO (P<0.001 vs. DG) and vitamin E (P=0.022 vs. DG), respectively (Table 2). The faecal total bacteria counts were similar among groups. FO significantly (P=0.032 vs. DG) increased the relative proportions (percentage of total bacteria) of

Plasma alanine aminotransferase activity

The plasma alanine aminotransferase level (μKat/l) was 0.20 (SE 0.03), 0.32 (SE 0.05) (P=0.016 vs. control), 0.22 (SE 0.02) and 0.17 (SE 0.02) in the control, DG, DG+FO and DG+vitamin E groups, respectively. The DG-induced change in alanine aminotransferase activity was normalised by FO (P>0.05 vs. control) and vitamin E (P>0.05 vs. control), respectively.

Fig. 1. Liver histology in Balb/cJ mice treated for 52 d with (a) vehicle control (saline, subcutaneous (s.c.)), (b) d-galactose (1.2 g/kg, s.c.), (c) d-galactose (1.2 g/kg, s.c.) + fructo-oligosaccharide (5 %, w/w) or (d) d-galactose + vitamin E (0.2 %, w/w). Livers were dissected after systematic perfusion with neutral formalin (n=4) for 5 min and then fixed in Bouin’s solution overnight. Tissues were processed for histological routine and stained with haematoxylin and eosin (original magnification, 200×). Scale bar represents 50 μm.
Table 2. Faecal total bacteria and Bifidobacterium counts of d-galactose-treated Balb/cJ mice.

(Mean values with their standard errors, n = 10)

<table>
<thead>
<tr>
<th></th>
<th>Bifidobacterium (log_{10} counts/g dry faeces)</th>
<th>Total bacteria (log_{10} counts/g dry faeces)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Control</td>
<td>6.65b</td>
<td>0.02</td>
</tr>
<tr>
<td>DG</td>
<td>6.57a</td>
<td>0.02</td>
</tr>
<tr>
<td>DG + FO</td>
<td>6.73c</td>
<td>0.02</td>
</tr>
<tr>
<td>DG + vitamin E</td>
<td>6.66b</td>
<td>0.02</td>
</tr>
</tbody>
</table>

FO, fructo-oligosaccharide; DG, d-galactose. **a**-**c** Mean values with unlike superscript letters within a column were significantly different (P < 0·05; ANOVA followed by the least significant difference test).

FO is easily incorporated into drinks. The elderly with poor oral function can obtain sufficient dietary fibre by taking this type of dietary supplement. The level of FO offered in the present study is equivalent to 25 g/d for adults whose daily dry food intake is 500 g. An adequate intake for total fibre in foods is set as 25 and 38 g/d for young women and men, respectively (131). Therefore, the dose of FO supplement used in the present study is applicable to adults, including the elderly.

In conclusion, the present study suggests that FO, besides being a prebiotic fibre, could prevent oxidative stress and fatty liver that occur during ageing.

Acknowledgements

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References
