Dietary Galacto-Oligosaccharides Mixture Can Suppress Serum Phenol and p-Cresol Levels in Rats Fed Tyrosine Diet

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Summary Phenols (phenol and p-cresol) are amino acid metabolites produced by intestinal bacteria. Some reports have demonstrated that the accumulation of phenols in the serum has toxic effects in renal failure patients. In this study, we found that phenols accumulated in the serum of rats given a tyrosine diet, and that dietary intake of a galacto-oligosaccharide mixture (GOS) suppressed the accumulation of phenols in serum. Rats were fed a basal diet, tyrosine diet (basal diet with 2.5% tyrosine) or GOS diet (tyrosine diet with 5% GOS) for 2 wk. The concentrations of phenols in the feces, cecal contents, serum and urine were determined. Concentrations of phenols in the serum, cecal contents and feces from rats fed the tyrosine diet were significantly higher than those in rats fed the basal diet. The concentrations of phenols in feces, cecal contents and serum, and urinary excretion in the GOS diet group were significantly lower than those in the tyrosine diet group. The pH of cecal contents was decreased by GOS intake. Furthermore, the serum concentrations of phenols were closely correlated with cecal concentrations. This finding suggested that concentrations of phenols in the serum reflected phenol production in the cecum contents. These results showed that dietary intake of GOS could modify the intestinal environment, and suppress the production of phenols in the intestinal tract and the accumulation of phenols in the serum. Thus, GOS may help improve the quality of life (QOL) of patients with renal failure.

Key Words phenol, p-cresol, tyrosine, galacto-oligosaccharide, rats

Phenols (phenol and p-cresol) are metabolites of aromatic amino acids (1), and are produced by intestinal bacteria in animals. It is assumed that an increase of undigested proteins in the lower small intestine enhances production of phenols. The excretion of phenols into the urine is also increased by a high consumption of meat protein (2) and tyrosine (3).

In renal failure patients, phenols accumulate in the blood because of insufficient excretion of waste into the urine. The accumulation induces various uremic symptoms (4–6), and p-cresol affects several biochemical, biological and physiological functions (7). Phenols in blood exist in the sulfate- and glucuronide-conjugated forms and unconjugated form. In particular, unconjugated (protein-bound and free) phenols have toxic effects in the body (8).

It has been reported that phenols accumulated in the blood of experimental uremic rats (9), whereas serum phenol levels were very low or undetectable in rats with normal renal function. In this study, we adopted supplementation of a tyrosine-rich diet to increase the serum levels of phenols in normal rats.

Galacto-oligosaccharides are produced from lactose by β-galactosidase. Galacto-oligosaccharides cannot be digested by human intestinal enzymes and serve as a substrate for endogenous colonic bacteria (10). Ingestion of galacto-oligosaccharides leads to a decrease of fecal pH, and increases of intestinal lactic acid bacteria (Bifidobacterium and Lactobacillus) (11, 12). Previous reports showed that ingestion of galacto-oligosaccharides with/without Bifidobacterium decreased the concentration of p-cresol in urine and feces in rats (13) and humans (11).

In this study, we used rats fed a tyrosine diet as an experimental model, and examined whether intake of galacto-oligosaccharides could suppress the accumulation of unconjugated phenols in blood.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats 6 wk of age were obtained from Charles River Japan Inc. (Yokohama, Japan). Rats were housed individually in stainless steel wire-bottomed cages (W260×D382×H200 mm) in an air-conditioned room at a temperature of 20–26°C, a 12-h light and dark cycle (lights on from 8:00 am to 8:00 pm) and 40–60% humidity, and allowed free access to food and water. Experimental diets were resupplied twice a week.

Composition of diets and galacto-oligosaccharide mixture (GOS). The basal diet consisted of 200 g casein, 150 g corn starch, 50 g cellulose powder, 50 g corn oil, 3 g DL-methionine, 2 g choline bitartrate, 500 g sucrose, 35 g mineral mixture (AIN-76 formulation) and 10 g vitamin mixture (AIN-76 formulation) per 1 kg of diet. The tyrosine diet contained tyrosine at the indicated concentrations in place of sucrose in the basal diet. The
GOS diet was prepared by addition of GOS at a final concentration of 5% to the tyrosine diet. GOS (Oligomate 55P; Yakult Yakuhin Kogyo, Tokyo, Japan) was composed of 59.1% galacto-oligosaccharides (18.2% disaccharides, 23.6% trisaccharides, 17.3% tetra- and hexa-saccharides), 21.6% lactose and 19.3% monosaccharide. GOSs were prepared from lactose using β-galactosidase from Bacillus circulans (14).

Experimental schedule

Exp. 1: Accumulation of serum phenols in rats by consumption of tyrosine diet: Twenty rats were fed the basal diet for 7 d. They were then randomly assigned to 4 groups of 5 rats with a similar mean body weight and food intake. Rats in each group were fed the 1, 2.5 or 5% tyrosine diet or the basal diet for 14 d.

Exp. 2: Suppressive effect of dietary GOS on serum concentrations of phenol and p-cresol in rats fed tyrosine diet: Eighteen rats were fed a commercial non-purified diet (MF; Oriental Yeast, Tokyo, Japan) for 7 d. They were then divided into 3 groups. Six rats were fed the basal diet during the experiment. Twelve rats were fed the 2.5% tyrosine diet for 9 d and then were randomly assigned to 2 groups of 6 rats with a similar mean body weight, food intake and urinary excretion of phenols. These rats were fed the 2.5% tyrosine diet with or without 5% GOS for 14 d. GOS was included in the tyrosine diet in place of sucrose. The GOS diet had approximately 3% lower energy content in comparison with the tyrosine diet, because 59.1% of GOS that was added to the diet at 5% was indigestible galacto-oligosaccharides. However, the rate of energy decrease of the GOS diet was less than the coefficient of variation of the diet intake in each group. Therefore, the difference in energy between the two diets was considered to have little influence on the study results.

This experimental design was approved by the Animal Experiment Committee of Yakult Central Institute, and the rats were managed according to the Guidelines for Care and Use of Experimental Animals.

Collection of urine, feces, blood and cecal contents.

Urine was collected for 24 h in a glass flask containing 1 N HCl (3 mL) using metabolic cages on day 12. Fresh feces were directly obtained from the rats on day 13. Blood was obtained from the abdominal aorta under anesthesia with diethyl ether on day 14. Then, the cecum was excised and dissected from the fat and mesentery, and weighed to determine the total weight. After the collection of cecal contents into a polypropylene tube, the cecal tissue was washed with saline, blotted and weighed. The weight of cecal contents was calculated by subtracting the cecal tissue weight from the total weight. The pH of cecal contents was measured directly with a compact pH meter (TPX-90i; Tokyo Chemical Laboratories Co. Ltd., Tokyo, Japan).

Sample preparation and measurement by HPLC.

Feces and cecal contents were diluted 10-fold with 0.1 M phosphate buffer (pH 5.5) (15) and homogenized. The suspensions were centrifuged at 3,000 rpm for 10 min, and the supernatant fluid was obtained. The urine was centrifuged at 2,000 rpm for 5 min, and the supernatant was stored. The supernatant fluid of feces and cecal contents, urine and serum were stored at −80°C until the measurement of phenols.

Unconjugated (protein-bound and free) phenols in feces, cecal contents and serum were measured by the modified HPLC method described by Niwa (16). Standard solutions were prepared at concentrations of 0.5–100 nmol/mL for phenol and p-cresol, p-Isopropylphenol at 0.5 μmol/mL, was used as an internal standard.

The total amounts of phenol and p-cresol (unconjugated and conjugated form) in the urine were measured with an acid-hydrolyzed preparation according to the method of Yoshikawa et al. (17).

The chromatography system consisted of a solvent degasser (L-7610), an auto-sampler (L-7200), a pump (L-7100), a fluorescence detector (L-7485), a column oven (L-7400) and an HPLC system manager (D-7000). These devices were obtained from HITACHI, Ltd., Tokyo, Japan. The routine column [F=411, 4.6 mm (i.d.)×150 mm, particle size 5 μm] was a ODS(C18) of Shodex (Tokyo, Japan). The HPLC conditions were the same as described by Niwa (16).

Statistical analysis. Data are expressed as the means±SD. The difference between which was evaluated by one-way analysis of variance, followed by multiple comparisons with the Tukey test and the statistical significance of the difference of the means was evaluated at the level of p<0.05. If the values were below the

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**Fig. 1.** Dose-response relationship between amount of tyrosine added to diet and serum concentrations of phenol (A) and p-cresol (B). Rats were fed a basal diet or 1, 2.5 or 5% tyrosine diet for 2 wk. Each value is expressed mean±SD. Values with different superscripts are significantly different at p<0.05.
Body weight and dietary intake
In Exp. 2, the body weight of rats in every group
were 50% of the basal diet weight. The dietary
intake was also 50% of the basal diet intake.

<table>
<thead>
<tr>
<th>Items</th>
<th>Basal diet group</th>
<th>Tyrosine diet group1</th>
<th>GOS diet group2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feces (nmol/g)</td>
<td>32±23a</td>
<td>(5/6)</td>
<td>164±100b</td>
</tr>
<tr>
<td>Cecal contents (nmol/g)</td>
<td>56±35a</td>
<td>(5/6)</td>
<td>804±215b</td>
</tr>
<tr>
<td>Serum (nmol/mL)</td>
<td>0.9±0.4a</td>
<td>(4/6)</td>
<td>17.9±7.0b</td>
</tr>
<tr>
<td>Urine (µmol/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before start1</td>
<td>1.3±0.5a</td>
<td>(6/6)</td>
<td>21.6±5.8b</td>
</tr>
<tr>
<td>After 2 wk</td>
<td>3.0±1.6a</td>
<td>(6/6)</td>
<td>52.7±13.0b</td>
</tr>
<tr>
<td>p-Cresol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feces (nmol/g)</td>
<td>77±66a</td>
<td>(6/6)</td>
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</tr>
<tr>
<td>Cecal contents (nmol/g)</td>
<td>5a</td>
<td>(0/6)</td>
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</tr>
<tr>
<td>Serum (nmol/mL)</td>
<td>0.5±0.0a</td>
<td>(1/6)</td>
<td>60.9±25.9b</td>
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<tr>
<td>Urine (µmol/d)</td>
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<td></td>
</tr>
<tr>
<td>Before start1</td>
<td>4.7±3.0a</td>
<td>(6/6)</td>
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<tr>
<td>After 2 wk</td>
<td>3.8±2.0a</td>
<td>(6/6)</td>
<td>176.9±57.8b</td>
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<tr>
<td>Cecal tissue weight (g)</td>
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<td>0.77±0.05a</td>
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<tr>
<td>Cecal content weight (g)</td>
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<td>2.84±0.50a</td>
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<tr>
<td>Cecal pH</td>
<td>6.91±0.06a</td>
<td></td>
<td>6.90±0.05a</td>
</tr>
</tbody>
</table>

The values are means±SD (n=6). Values with different superscripts are significantly different at p<0.05.
1 Rats fed 2.5% tyrosine-supplemented diet.
2 Rats fed 2.5% tyrosine-supplemented diet with 5% GOS.
3 Values in parentheses are No. of rats with detectable level/No. of tested rats. If the determined value was below the detection limit, the value of the detection limit (5 nmol/g for feces and cecal contents and 0.5 nmol/mL for serum) was used.
4 Phenols in urine are expressed as total substances (unconjugated and conjugated substances).
5 Rats in tyrosine diet group and GOS diet group fed tyrosine diet for 9 d before the start.

RESULTS
Accumulation of serum phenols in rats fed tyrosine diet
In Exp. 1, the serum concentrations of phenol and p-cresol increased dose-dependently according to the content of tyrosine (1–5%) added in the diet (Fig. 1). Compared with the basal diet group, the serum concentrations of phenol and p-cresol in the 2.5% and 5% tyrosine diet groups increased. Thus, a diet supplemented with 2.5% tyrosine was chosen to examine the suppressive effect of GOS on accumulation of serum phenols.

Relationship between serum and cecal concentrations of phenols
The relationship between serum and cecal concentrations of phenol or p-cresol in the basal diet and the 1, 2.5 and 5% tyrosine diet groups was examined (Fig. 2). As for phenols, their serum concentrations were closely correlated with their cecal concentrations. The correlation coefficient between serum and cecal phenol or p-cresol concentrations was quite similar to each other.

Body weight and dietary intake
In Exp. 2, the body weight of rats in every group...
gradually increased during the experimental period. Diet intake in the basal diet group, tyrosine diet group and GOS diet group during the experimental period was $23.1 \pm 1.4$, $22.8 \pm 1.3$ and $22.4 \pm 2.3$ g/d, respectively. The mean GOS intake during the experimental period was 1.12 g/d in the GOS diet group.

**Phenols in rats fed basal diet and tyrosine diet**

Results are shown in Table 1. Phenol and p-cresol concentrations in the feces, cecum and serum were significantly higher in tyrosine diet group than these in the basal diet group. Similarly urinary excretion was also elevated in rats fed the tyrosine-supplemented diet.

**Effect of GOS intake on phenol concentrations and intestinal conditions**

Fecal, cecal and serum concentrations and urinary excretion of phenols were lower in the GOS diet group than in the tyrosine diet group. Cecal tissue weight and cecal contents significantly increased in the GOS diet group, while cecal pH was rather lower in the tyrosine diet group (Table 1).

**DISCUSSION**

When rats were fed diets supplemented with excess tyrosine at various concentrations (1−5%), the concentrations of phenols in serum increased in a dose-dependent manner. In particular, p-cresol increased exponentially. The increment was high in both 2.5% and 5% tyrosine diet groups relative to the basal diet group. This is the reason why we chose a common 2.5% tyrosine diet for comparison with the GOS group.

As shown in Fig. 2, serum concentrations of phenols were closely correlated with cecal concentrations, but the correlation between phenol levels in serum and feces was less than that in serum and cecum contents (data not shown). It is known that dietary tyrosine is converted to phenols by intestinal bacteria, which are absorbed into the blood. Serum phenols would have reflected their enhanced production in the cecum.

In Exp. 2, fecal and cecal concentrations of phenols increased much more in the 2.5% tyrosine diet group than in the basal diet group; their increments were 4-fold and 12-fold respectively, unlike a 19-fold increase in fecal p-cresol level (Table 1). Cecal p-cresol level in the tyrosine diet group was very high (3.635±1.022 mol/g) irrespective of the absence of detection of p-cresol in the basal diet group.

As shown in Table 1, GOS intake markedly suppressed the production of phenols in the intestine and its concomitant transfer in the blood and excretion into the urine. At that time, we observed considerable increases in cecal tissue weight and cecal contents as well as a decrease in cecal pH in rats fed the GOS diet. Other researchers have reported findings in the case of ingestion with fructo-oligosaccharides or xylo-oligosaccharides (18). It has been demonstrated that galacto-oligosaccharides cause an increase in some short-chain fatty acids (SCFA) such as acetate and propionate in in vitro culture with human feces (19). Morishita et al. reported that the intake of galacto-oligosaccharides decreased pH and increased SCFA in the cecal contents of rats (20). The lower pH and high carbohydrate availability led to a marked reduction in dissimilatory metabolism of aromatic amino acids (21). Therefore, it is suggested that the intake of GOS might modify intestinal microflora and suppress production of phenols in the cecum. Additionally, the GOS intake suppressed urinary excretion of phenols (conjugated and unconjugated forms) for 24 h. It is highly probable that GOS suppresses the total amount of phenols absorbed from the intestinal tract into the body and thereby lessens accumulation of phenols in the serum.

Phenols, which are produced by intestinal bacteria, exist mainly in the unconjugated form in the intestinal tract. Phenols are absorbed from the intestines, and are metabolized in the liver. Phenols in the blood exist in sulfate- and glucuronide-conjugated forms or unconjugated form. The unconjugated form has toxicity in the body in comparison with the conjugated forms. Some reports describe unconjugated p-cresol as being injurious to several biochemical, biological and physiological functions (7). It has been known that p-cresol plays a role in the immunodeficiency of uremia because it inhibits the function of polymorphonuclear cells and macrophages in vitro (8, 22). Therefore, uremic patients have misgivings about pathogenic infections caused by the impairment of innate immune resistance.

In renal failure patients treated with dialysis, intestinal putrefactive substances, such as phenols and indican, are maintained at higher serum levels than normal. To maintain the health of these patients, it is important to suppress the production and absorption of intestinal putrefactive substances, because of their insufficient urinary excretion. It has been reported that administration of lactic acid bacteria could decrease fecal p-cresol in uremic patients undergoing dialysis (23). In this study, the intake of GOS suppressed the accumulation of phenols in the serum of tyrosine-fed rats. It is possible that the serum phenol level of uremic patients undergoing dialysis could be reduced by administration of GOS. With developments in dialysis technology for renal insufficiency, uremic patients are now undergoing long-term artificial dialysis. The control of life-style factors such as diet is important to prevent various complications. Supplementation with GOS may help to improve the quality of life of patients with renal failure.

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