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Effects of synbiotic treatment on serum level of *p*-cresol in haemodialysis patients: a preliminary study

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Abstract

Background. *para*-Cresol, which is present in the blood mainly as *p*-cresyl sulphate, is a protein-bound uraemic toxin that is produced in the intestine by certain intestinal bacteria, and its production is affected by various intestinal environmental factors. Patients with end-stage renal disease who are undergoing haemodialysis (HD) often have defective bowel function leading to abnormal defecation. Since treatment with synbiotics (SYN), which are a combination of probiotics and prebiotics, is reported to improve bowel habit, we examined the effects of SYN on the serum *p*-cresol level in HD patients.

Methods. Nine HD patients received SYN (*Lactobacillus casei* strain Shirota and *Bifidobacterium breve* strain Yakult as probiotics and galacto-oligosaccharides as prebiotics) three times a day for 2 weeks. The duration of the study was 4 weeks (2 weeks of pretreatment observation and 2 weeks of treatment). The subjects were asked to complete a questionnaire about their bowel habits (defecation frequency, stool quantity, stool form and ease of defecation) during the study period. Serum *p*-cresol levels before and after SYN treatment were determined.

Results. According to the questionnaire conducted during the pretreatment observation period, HD patients with a high serum *p*-cresol level tended to have hard stools with difficulty in defecation. With SYN treatment, stool quantity increased significantly and hard, muddy or soft stools tended to be replaced by normal ones. The serum *p*-cresol level also decreased significantly.

Conclusions. It was found that uraemic toxin, *p*-cresol, was associated with constipation and that SYN treatment resulted in normalization of bowel habits and a decrease of serum *p*-cresol levels in HD patients. Therefore, SYN treatment may be anticipated to reduce the toxic effect of *p*-cresol in HD patients.

Keywords: constipation; haemodialysis; *p*-cresol; *p*-cresyl sulphate; synbiotic

Introduction

Patients with end-stage renal disease (ESRD) who have loss of kidney function accumulate a variety of substances in the body which can cause uraemia. These substances may be classified into three groups (small water-soluble solutes, protein-bound solutes and middle-molecule solutes) according to their biochemical properties [1]. Haemodialysis (HD) cannot effectively eliminate protein-bound solutes as opposed to small water-soluble solutes, and the accumulation of protein-bound uraemic toxins such as *p*-cresol and indoxyl sulphate (IS) is suggested to be related to complications and mortality in HD patients [2]. In particular, *p*-cresol, which exists mainly as *p*-cresyl sulphate in the blood [3], is associated with infection, cardiovascular disease and mortality in HD patients [4–6]. An *in vitro* study confirmed that the biological/biochemical activity of *p*-cresyl sulphate is related to the development of cardiovascular disease [7,8].

Phenols (*p*-cresol and phenol) and indole, the precursors of IS, are nitrogenous metabolites produced by intestinal bacteria from tyrosine and tryptophan, respectively. Their production is influenced by the intestinal environment, such as intestinal flora and pH [9]. Prolonged transit time in the colon, abnormal intestinal flora and abnormal defecation (especially constipation) are frequently observed in HD patients [10–12].

Inhibition of the production of the precursors of uraemic toxins is one of the effective ways to reduce the accumulation of uraemic toxins in HD patients. Probiotics, which have beneficial effects on the body, prebiotics including indigestible polysaccharide, which encourage the growth of beneficial intestinal bacteria, and synbiotics (SYN), which combine probiotics and prebiotics, improve the intestinal environment and bowel habit and can also reduce intestinal nitrogenous metabolite concentrations [13,14]. In particular, SYN treatment is extremely effective for improving the intestinal environment in various medical conditions [15].

While abnormal defecation is one of the symptoms that reduces the quality of life in HD patients, it is also a non-invasive indicator of bowel function and the condition of the intestinal flora. In this study, we tried SYN treatment in HD patients. We focused on the relation between the serum concentrations of uraemic toxins (*p*-cresol, phenol and IS) and bowel habit and examined the effectiveness of SYN treatment on them.

Materials and methods

Subjects

Nine outpatients (three male and six female) undergoing HD treatment three times a week at Toyoda Clinic (Hino, Tokyo, Japan) were enrolled in the study. Their primary diseases were diabetic nephropathy (five patients), chronic glomerulonephritis (two patients), renal dysplasia (one patient) and nephrosclerosis (one patient). The subjects' characteristics were as follows: age [median (min–max)]: 63 (38–82) years; HD duration: 3.4 (0.2–5.9) years; body mass index, calculated as body weight (kg) / height $(m)^2$: 21.5 (16.3–28.3); urea nitrogen: 61 (40–85) mg/dL; creatinine: 9.0 (5.3–12.6) mg/dL; calcium: 9.0 (8.0–9.6) mg/dL; phosphorus: 4.2 (3.5–7.1) mg/dL; total protein: 6.8 (6.6–7.6) g/dL and albumin: 3.7 (3.1–3.9) g/dL.

Study design

The subjects received SYN (1 packet of Yakult BL Seichoyaku® as probiotics and 4 g of Oligomate 55N® as prebiotics) three times a day for 2 weeks. Yakult BL Seichoyaku®, a drug for controlling intestinal function, was purchased from Yakult Honsha Co., Ltd, Tokyo, Japan, and Oligomate 55N® was obtained from Yakult Pharmaceutical Co., Ltd, Tokyo, Japan. One packet of Yakult BL Seichoyaku contains 1×10^8 *Lactobacillus casei* strain Shirota (LcS) and *Bifidobacterium breve* strain Yakult (BbY), and 4 g of Oligomate 55N contains 1.67 g or more galacto-oligosaccharides (GOS) and <1.36 g of lactose and monosaccharide. The study duration was 4 weeks: a 2-week pretreatment observation period and a 2-week treatment period. The subjects were asked to complete a questionnaire every day regarding defecation and abdominal symptoms during the study period. Blood concentrations of *p*-cresol, phenol and IS were measured before and after SYN treatment. The subjects did not have to restrict their everyday diet, medication or daily activities. Five subjects chronically used acid reducers, five used laxatives, two used a phosphate binder (sevelamer hydrochloride) and one used medication containing live lactic acid bacteria. None of the subjects used antibiotics during the study period.

This study was conducted according to the principles of the Declaration of Helsinki of the World Medical Association and was approved by the ethics committee of Hachioji Medical Center of Tokyo Medical University. All participants gave written informed consent.

Questionnaire on bowel habit and abdominal symptoms

During the study, subjects were asked to evaluate via a questionnaire, their own defecation frequency (times per week), stool quantity (a score of 1 is equivalent to 1.5 cm in diameter × 5 cm in length per week), stool form [scored using the modified Bristol stool form scale [16]: 1 = very hard stool (small hard lumps), 2 = hard stool (hard sausage shape), 3 = normal stool (sausage to banana shape), 4 = soft stool, 5 = muddy stool, 6 = watery stool], ease of defecation (1 = difficult, 2 = easy, 3 = very easy) and abdominal symptoms [frequency of upper abdominal pain, lower abdominal pain, borborygmus, flatulence and flatus were scored (1 = frequent, 2 = occasional, 3 = almost never)], and the average scores before and after SYN treatment were calculated after the study period.

Biochemical analyses

Blood biochemical parameters were measured by standard analytical methods using automatic analysis equipment before SYN treatment. Normal ranges of urea nitrogen, creatinine, calcium, phosphorus, total protein and albumin were 8–22 mg/dL, 0.6–1.0/0.4–0.8 mg/dL (male/female), 8.5–11.0 mg/dL, 2.5–4.5 mg/dL, 6.5–8.2 g/dL and 3.7–5.2 g/dL, respectively.

Serum *p*-cresol and phenol levels were measured by a protocol with modification of the fluorescent HPLC method of Niwa [17]. To quantify total *p*-cresol and phenol, serum was hydrolysed with concentrated hydrochloric acid (at 100°C for 1 h) [18]. An F-411 analytical column (150 mm × 4.6 mm [i.d.]; Shodex®, Tokyo, Japan), 0.1% H_3PO_4/acetonitrile (70/30) elutant, *p*-propylphenol as internal standard and a fluorescence detector (EX: 260 nm, EM: 305 nm) were used to detect both substances. Recently, it has been reported that *p*-cresyl sulphate is completely hydrolysed to *p*-cresol by heat-acid deproteinization, and *p*-cresol concentration in hydrolysed serum can be equated to the serum concentration of *p*-cresyl sulphate [19].

Serum IS level was measured according to the fluorescent HPLC method [20]. A CAPCELL PAK MF Ph-1 SG80 analytical column (150 mm × 4.6 mm [i.d.]; Shiseido, Tokyo, Japan), 0.1 M KH_2PO_4/tetrahydrofuran (95/5) elutant and a fluorescence detector (EX: 295 nm, EM: 390 nm) were used to detect IS.

Statistical analysis

Spearman's rank correlation test was used to analyse the correlation between serum levels of *p*-cresol, phenol and IS and each parameter of bowel habit. Wilcoxon signed-rank sum test was used for comparison of values before and after SYN administration.

Results and discussion

The results of the questionnaires during the pretreatment observation period (n = 9) showed that the HD patients

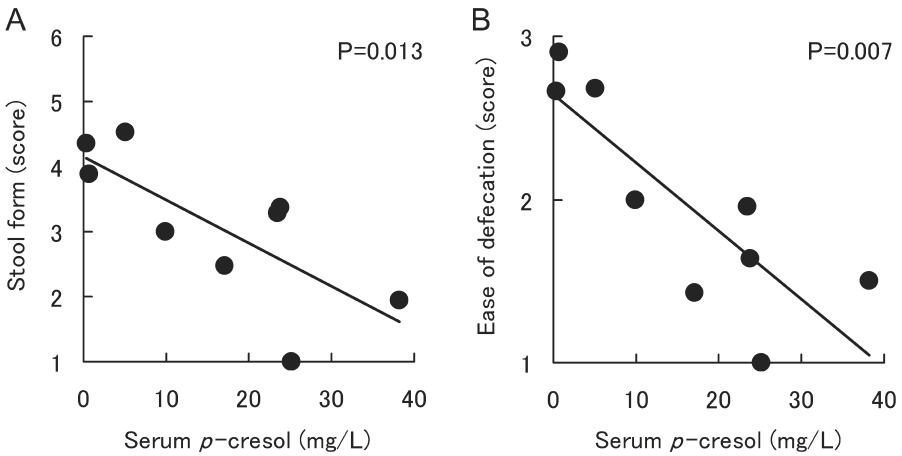

Fig. 1. Rank correlation between serum *p*-cresol and bowel habit parameters [stool form (**A**) and ease of defecation (**B**)] in nine patients. Scores of stool form are 1 (very hard stool)–6 (watery stool). Scores of ease of defecation are 1 (difficult), 2 (easy) and 3 (very easy). P-values were obtained using Spearman's rank correlation test.

Table 1. Effects of SYN treatment on serum levels of uraemic toxins, bowel habit and abdominal symptoms

Parameters	Before SYN treatment (n = 9)	Before SYN treatment (n = 7)	After SYN treatment (n = 7)	P
Uraemic toxins				
p-Cresol (mg/L)	16.0, 17.1 (0.3–38.2)	16.8, 17.1 (0.3–38.2)	13.4, 14.2 (0.4–27.1)	0.031
Phenol (mg/L)	5.1, 3.7 (1.0–12.4)	5.5, 3.7 (1.0–12.4)	6.6, 4.6 (0.5–16.3)	n.s.
Indoxyl sulphate (mg/L)	26.5, 23.9 (6.9–52.4)	28.6, 32.2 (6.9–52.4)	26.8, 30.1 (6.3–45.8)	n.s.
Bowel habit				
Defecation frequency (times/week)	9.1, 8.5 (1.5–22.5)	7.4, 8.5 (1.5–12.0)	7.2, 8.5 (2.5–10.0)	n.s.
Stool quantity (score[a])	22.8, 18.0 (4.5–50.0)	22.6, 18.0 (4.5–50.0)	25.6, 19.5 (8.9–51.0)	0.031
Stool form (score[b])	3.1, 3.3 (1.0–4.5)	3.3, 3.3 (1.9–4.5)	3.5, 3.5 (2.8–4.3)	n.s.
Ease of defecation (score[c])	2.0, 2.0 (1.0–2.9)	2.0, 2.0 (1.4–2.7)	1.9, 1.9 (1.4–2.5)	n.s.
Abdominal symptoms				
Upper abdominal pain (score[d])	2.8, 3.0 (2.1–3.0)	2.8, 3.0 (2.1–3.0)	2.8, 3.0 (2.1–3.0)	n.s.
Lower abdominal pain (score[d])	2.7, 2.8 (2.3–3.0)	2.7, 2.8 (2.3–3.0)	2.7, 2.7 (1.9–3.0)	n.s.
Borborygmus (score[d])	2.5, 2.4 (2.0–3.0)	2.5, 2.4 (2.0–3.0)	2.5, 2.8 (1.8–3.0)	n.s.
Flatulence (score[d])	2.5, 2.6 (1.6–3.0)	2.6, 2.9 (1.6–3.0)	2.4, 2.6 (1.2–3.0)	n.s.
Flatus (score[d])	2.2, 2.1 (1.0–3.0)	2.2, 2.1 (1.0–3.0)	2.1, 2.0 (1.0–3.0)	n.s.

Values are shown as mean, median (min–max). P-values were obtained using Wilcoxon signed-rank test (significant difference between before and after SYN treatment; n = 7). n.s.: not significant.
[a]Score 1 is equivalent to 1.5 cm (diameter) × 5 cm (length) per week.
[b]Scores 1 (very hard stool)–6 (watery stool).
[c]Scores 1 (difficult), 2 (easy) and 3 (very easy).
[d]Scores 1 (frequent), 2 (occasional) and 3 (almost never).

with a high serum *p*-cresol level had hard stools and difficulty in defecating (Figure 1). However, the serum phenol and IS levels did not correlate with any parameter of bowel habit (data not shown). These findings indicate that *p*-cresol is a uraemic toxin related to bowel habit (especially constipation).

Serum concentration (min–max) of *p*-cresol in our study was 3.2–353.5 μM (0.3–38.2 mg/L). Previously reported serum level of *p*-cresyl sulphate in healthy volunteers and HD patients was 3.65–56.06 and 3.09–439.79 μM, respectively [19], which was consistent with our data. *p*-Cresol, which is present mainly as *p*-cresyl sulphate in human blood, is difficult to remove by HD and accumulates in the blood of HD patients. However, serum *p*-cresol level of individuals showed great variation. This fact indicates that the serum level in HD patients is sensitively influenced by the *p*-cresol level in the intestine through production in the intestine and intestinal environmental factors.

One female subject discontinued SYN treatment during the study through her own choice. Another female subject took indigestible dextrin and a fermented bifidus milk drink daily, but she stopped taking these products during the treatment period. We excluded these two patients and evaluated the effect of SYN treatment on the serum concentration of uraemic toxins and bowel habit based on the data of the remaining seven subjects. One male subject usually took medication containing live lactic acid bacteria for improvement of intestinal condition and continued taking this during the study. As we planned to do this study under regular medication, his data were not excluded.

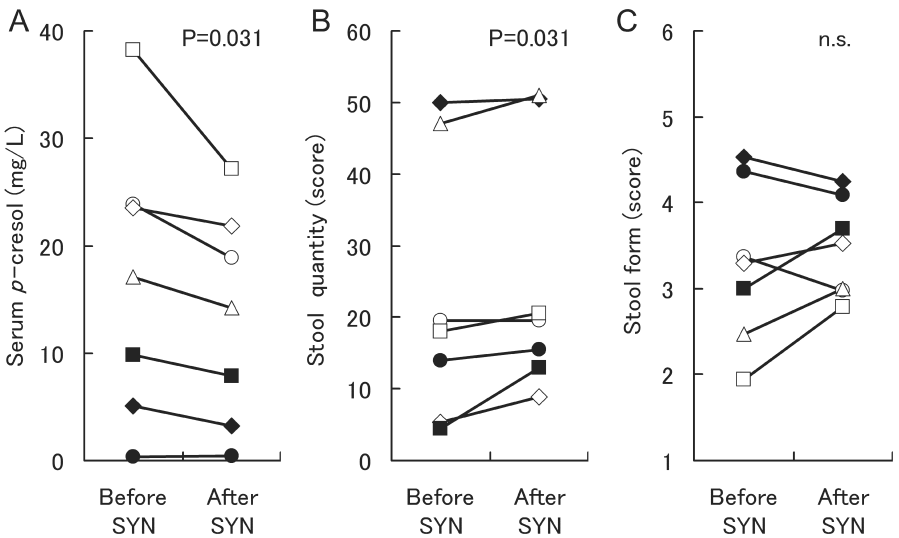

Fig. 2. Effects of SYN treatment on serum *p*-cresol (**A**), stool quantity (**B**) and stool form (**C**) in seven patients. Score 1 of stool quantity is equivalent to 1.5 cm (diameter) × 5 cm (length) per week. Scores of stool form are 1 (very hard stool)–6 (watery stool). P-values were obtained using Wilcoxon signed-rank sum test. Each symbol in (A), (B) and (C) was represented to correspond to individual data, respectively. n.s.: not significant.

SYN treatment did not appear to affect serum phenol and IS concentrations, but it significantly reduced the serum *p*-cresol level (Table 1, Figure 2). In healthy people, it was reported that LcS or BbY administration reduces urinary *p*-cresol excretion [21] and that GOS reduces the faecal *p*-cresol concentration [22]. The reduction in the serum *p*-cresol level with SYN administration was considered to result from inhibition of *p*-cresol production in the intestine.

In addition, stool quantity significantly increased with SYN treatment (Table 1, Figure 2). Hard or muddy to soft stools tended to change to normal stools (Figure 2). In three subjects with a defecation frequency of less than seven times/week, the frequency increased, and in one subject with a defecation frequency of 12 times/week, it decreased. Moreover, SYN treatment did not adversely affect abdominal symptoms including abdominal pain. GOS is reported to soften stools and increase defecation frequency in healthy people who have a tendency to suffer from constipation [23]. However, no significant change in stool form or defecation frequency with SYN administration was observed in this study. This may have been due to two reasons: most subjects controlled their defecation with laxatives, and not all the subjects had a background of hard stools. These results demonstrated that SYN treatment tends to increase faecal volume and normalize stool form. It is believed that this normalization of bowel habit affects intestinal nitrogen metabolism and triggers a reduction in serum *p*-cresol concentration.

It has been reported that serum *p*-cresol level in diabetic HD patients is significantly higher than that of non-diabetic HD patients [5]; also, diabetic patients have a disorder of gastrointestinal motility due to autonomic neuropathy, abnormal microflora and constipation [24]. In the present study, the three subjects with the highest serum *p*-cresol level were diabetic HD patients. It is suggested that constipation due to gastrointestinal motility disorder may be a factor in the increase in serum *p*-cresol level in diabetic HD patients. However, the relation between effectiveness of SYN and diabetes is not yet clear.

HD patients usually receive various concomitant therapy, especially treatment with laxatives affecting bowel habit. Our results showed that SYN could improve bowel habit and decrease the serum *p*-cresol level in HD patients without restriction of concomitant therapy including laxatives. Limitations to this preliminary study include the small sample size, lack of a control group and no data of *p*-cresol generation rate and nutrition intake. This preliminary study was conducted in patients with an HD history of <6 years. To evaluate the effectiveness of SYN treatment in HD patients, adequately designed studies including long-term HD patients who have problems with uraemic toxin accumulation and complications are necessary to be conducted under the study condition excluding the limitations of this preliminary study. We plan to carry out such work in the future.

In conclusion, the results of this preliminary study demonstrated that *p*-cresol is a constipation-related uraemic toxin, and SYN treatment could normalize the bowel habit of HD patients and lower their serum *p*-cresol level. Therefore, SYN treatment may prevent the accumulation of *p*-cresol, and the resulting toxic effects in HD patients may be reduced.

Conflict of interest statement. None declared.

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