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From the Clinic

Oral administration of *Bifidobacterium longum* in a gastro-resistant seamless capsule decreases serum phosphate levels in patients receiving haemodialysis

When kidney function decreases, phosphate (P) *per se* can initiate and promote hyperparathyroidism and vascular calcification. Therefore, hyperphosphataemia is a widely recognized risk factor for mortality and cardiovascular disease in patients with chronic kidney disease (CKD) [1]. Despite the introduction in the market of new P-binders, such as sevelamer hydrochloride and lanthanum carbonate, the control of hyperphosphataemia in haemodialysis (HD) patients remains difficult because of adverse effects such as constipation and faecal impaction, drug compliance and metal accumulation. We here report an interesting observation that serum P levels can be reduced by modifying the intestinal flora with probiotic administration. This may suggest a new potential approach to better control hyperphosphataemia in HD patients.

In patients receiving HD, intestinal aerobic bacteria, such as enterobacteria and enterococci, occur in numbers ~100 times higher than those in healthy subjects. These aerobes produce putrefactive substances [2]. Oral administration of antibiotic-resistant lactic acid bacteria for 4 weeks can restore the composition of disturbed microflora characterized by overgrowth of aerobes and decreased levels of serum uraemic toxins, including indicant, in HD patients [2].

When administered orally, *Bifidobacteria* cannot survive exposure to gastric juice before they reach the intestine. In order to enable *Bifidobacteria* to reach the intestine, a colony-forming unit of *Bifidobacterium longum* JBL01, a strain of human *Bifidobacteria*, measuring 2.0×10^9, combined with 0.11 g of oligosaccharides (lactulose and raffinose) as a stimulant to bacteria proliferation, were encapsulated in a gastro-resistant seamless capsule (B capsule, MorishitaJintan Co., Ltd, Osaka, Japan). *Bifidobacteria* can survive in this gastro-resistant capsule even in a solution of 1.2 pH at 37°C for 120 min. In contrast, *Bifidobacteria* without the capsule (a powder formulation) cannot be detected immediately after mixing with the solution [3]. Moreover, significant decreases in serum levels of indoxyl sulphate and homocysteine were observed by oral administration of *B. longum* in the gastro-resistant capsule, but not by the administration of *B. longum* without the capsule in HD patients [4].

Thus, to improve the composition of disturbed microflora and alleviate faecal impaction, B capsules containing *B. longum* JBL01 were administered orally once daily for 4 weeks in 15 patients receiving HD [age: 62.2 ± 9.8 years; 10 males, 5 females; 5 diabetes mellitus (DM), 10 non-DM; HD duration: 9.3 ± 7.0 years; serum albumin levels: 3.9 ± 0.3 g/dL; serum corrected calcium (Ca) levels: 9.1 ± 0.8 mg/dL; serum P levels: 6.7 ± 0.6 mg/dL; serum intact parathyroid hormone (PTH) levels: 363 ± 221 pg/mL]. Results were compared with those of 16 HD patients who did not receive B capsules as a control group (age:58.1 ± 15.6 years; 10 males, 6 females; 3 DM, 13 non-DM; HD duration: 9.1 ± 6.8 years; serum albumin levels: 3.8 ± 0.3 g/dL; serum-corrected Ca levels: 9.0 ± 0.9 mg/dL; serum P levels: 7.0 ± 0.8 mg/dL; serum intact PTH levels: 219 ± 124 pg/mL). No patient had undergone treatment with *B. longum* preparations before participating in this study. The dose of drugs affecting P metabolism were fixed through the study period in the control group (Ca carbonate: 2.79 ± 1.44 g, $n = 14$; sevelamer hydrochloride: 3.20 ± 2.94 g, $n = 5$; calcitriol injection: 1.67 ± 0.24 μg/week, $n = 3$; maxacalcitol: 15.0 ± 4.1 μg/week, $n = 3$; oral alfacalcidol: 0.5 μg, $n = 2$, oral calcitriol: 0.38 μg, $n = 2$) and in the B capsule-treated group (Ca carbonate: 2.50 ± 0.71 g, $n = 12$; sevelamer hydrochloride: 3.75 ± 0.53 g, $n = 4$; calcitriol injection: 2.75 μg/week, $n = 2$; maxacalcitol: 12.5 ± 8.5 μg/week, $n = 4$; oral alfacalcidol: 0.25 ± 0 μg, $n = 3$, oral calcitriol: 0.25 μg, $n = 1$). No patients received lanthanum carbonate or cinacalcet hydrochloride in either group. All data are expressed as mean ± SD or without SD in $n = 1$ or 2.

Oral administration of B capsules for 4 weeks had no significant effect on defecation frequency or faecal hardness determined by the Bristol stool form scale (data not shown). The explanation for this result may be related to the low incidence of obstinate constipation from the beginning in this study (mean defecation frequency: 0.9/day). Serum P levels unexpectedly decreased significantly in the second and fourth weeks due to oral administration of the B capsule (Figure 1). Serum P levels remained unchanged in the control group. Subsequently, serum P levels returned to original levels 2 weeks after the completion of oral treatment. No significant changes were found in other serum parameters in the B capsule-treated group compared with baseline data and with the control group (data not shown).

The main mechanism for a decrease in serum P levels during B capsule treatment is its ability to lower intestinal pH levels. In the faeces of HD patients, pH levels and ammonia concentrations are elevated due to bacteria-mediated hydrolysis of urea [4]. High levels of ammonia are responsible for elevated pH in faeces and lead to overgrowth of aerobic and putrefactive bacteria. *Bifidobacteria* can ferment carbohydrates to produce acetic acid and lactic acid, resulting in acidification of the intestinal lumen [4]. Low pH levels in the intestinal milieu inhibit the growth of aerobic and putrefactive bacteria, thus normalizing the intestinal flora. In another study, the administration of *B. longum* decreased faecal pH levels, and pretreatment levels were restored 1 week after the completion of treatment [5]. Decreased intestinal pH levels due to B capsule treatment may increase the ionization of intestinal Ca. Ca^{2+} binds with intestinal P ions as an intrinsic P binder. In this study, increased intestinal Ca^{2+} did not lead to elevated serum Ca levels. Ca^{2+} may be immediately used in the formation of calcium phosphate in the intestine, and thus not absorbed. Further studies measuring faecal pH, P and Ca excretions will examine this as a possible mechanism in B capsule-treated patients.

In this study, although the number of faecal bacteria was not determined during B capsule treatment, the decrease in serum P levels cannot be due to increased

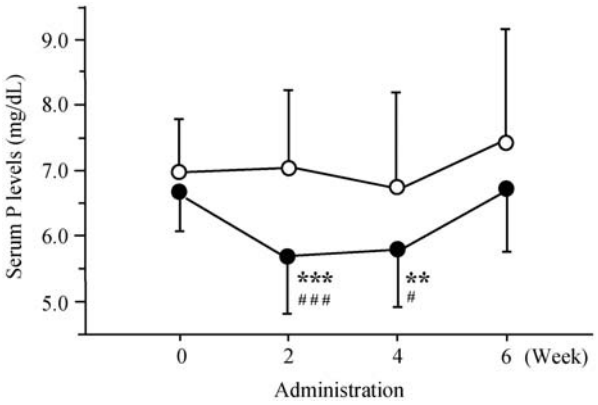

Fig. 1. Effect of once-daily oral administration of encapsulated *B. longum* JBL01 with oligosaccharides (B capsule) for 4 weeks on serum P levels in patients on haemodialysis. Open circle: untreated control group ($n = 16$); filled circle: B capsule-treated group ($n = 15$). Data are expressed as mean ± SD. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus baseline data (paired *t*-test). $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ versus the control group (Student's *t*-test).

uptake of P into the intestinal *B. longum* for their proliferation. Because feeding of *B. longum* increases their number, but does not affect the total number of bacteria through a reduction in aerobes in the faeces of healthy subjects [5]. In addition, increased faecal volume and water content are not responsible for low serum P levels, as evidenced by unchanged defecation frequency and faecal hardness among the patients in the present study.

It is increasingly recognized that uraemic toxins originating from intestinal microbial metabolism may contribute to the deterioration of renal function, cardiovascular calcification, metabolic bone disease and mortality in CKD/HD patients [6, 7]. The administration of *Bifidobacteria* can decrease the serum concentrations of indoxyl sulphate and P-cresol in HD patients [4, 7]. In addition, *Bifidobacteria* produce vitamin B12 and folate, which can normalize serum homocysteine levels in HD patients [3]. This is an important property since most HD patients show hyperhomocysteinaemia, which is a risk factor for cardiovascular disease. Although further investigation is clearly required to clarify the mechanism for the P-reducing effect of the B capsule, *Bifidobacteria* preparation is a simple and safe treatment for persistent hyperphosphataemia in patients receiving HD. Further studies may identify additional potential benefits of probiotic treatments in CKD/HD patients [6, 7].

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References

1. Palmer SC, Hayen A, Macaskill P *et al*. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. *J Am Med Assoc* 2011; 305: 1119–1127
2. Hida M, Aiba Y, Sawamura S *et al*. Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. *Nephron* 1996; 74: 349–355
3. Taki K, Takayama F, Niwa T. Beneficial effects of Bifidobacteria in a gastroresistant seamless capsule on hyperhomocysteinemia in hemodialysis patients. *J Ren Nutr* 2005; 15: 77–80
4. Takayama F, Taki K, Niwa T. *Bifidobacterium* in gastro-resistant seamless capsule reduces serum levels of indoxyl sulfate in patients on hemodialysis. *Am J Kidney Dis* 2003; 41: S142–S145
5. Benno Y, Mitsuoka T. Impact of *Bifidobacterium longum* on human fecal microflora. *Microbiol Immunol* 1992; 36: 683–694
6. Evenepoel P, Meijers BK, Bammens BR *et al*. Uremic toxins originating from colonic microbial metabolism. *Kidney Int* 2009; 114: S12–S19
7. Schepers E, Glorieux G, Vanholder R. The gut: the forgotten organ in uremia? *Blood Purif* 2010; 29: 130–136

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