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Research Article

Short-Term Effects of Pre/Probiotics on P-Cresol and Indoxyl-Sulphate Serum Concentrations During the Various Stages of Chronic Kidney Disease

Abstract

Background: The uremic syndrome is provoked by a progressive number of compounds that are normally excreted by kidneys in healthy individuals. Indoxylsulphate (IXS) and p-cresylsulphate (PCS), have been found increased in subjects with end stage renal disease (ESRD) creating great harm to biological systems; these uremic toxins come from the intestinal bacterial fermentation of the proteins. The aim of our study is to evaluate the short-term effects after an administration of pre / probiotics in CKD patients, regarding the production and then the serum concentrations of free IXS and PCS (i.e. non-protein bound fraction) and total IXS and PCS (i.e. sum of unbound and protein bound fraction).

Methods: In our study, 26 patients with CKD stage 2-5 associated with hypertension and / or diabetes mellitus type 2 were enrolled, and administered with 2 g/day dose of pre-probiotics for four months: mixed oligofructose (prebiotic component) + Lactobacillus acidophilus and Bifidobacterium longum (probiotic component). In all patients, at the beginning of the study, kidney function tests, glucose metabolism, PTH and blood uric acid were evaluated. Free and total PCS and IXS were also measured. 20 control subjects with normal renal function were considered in relation to the same parameters.

After 4 months 19 patients were re-evaluated in relation to the same parameters. Statistical differences were studied using the Student-t paired and unpaired tests.

Results: The baseline values of IXS and PCS of the 26 patients were significantly higher compared with the normal subjects and importantly increased with the transition to the higher stage of CKD. The values in stage 2 - 3 CKD were significantly lower in respect to stage 4 - 5 CKD. Higher mean values of IXS and PCS in 12 diabetic subjects were highlighted, although not statistically significant compared to 14 hypertensive non-diabetic patients.

The data after the use of pre - probiotics in 19 patients that completed the treatment protocol (5 patients were out of the study for non-compliance of the processing and 2 patients for dialysis entrance), showed increased concentrations of free and total IXS and PCS. Considering renal function, the use of pre - probiotics increased the concentration of free and total PCS and IXS in all conditions, while remaining significantly higher in patients at stage 4-5 rather than in the ones at the stage 2-3. The use of pre - probiotics increased the IXS and PCS serum concentrations, remaining significantly higher in diabetics rather than in hypertensive patients. In all periods, both baseline and after the uptake of pre-probiotics, the other measured parameters didn't change except serum PTH that decreased significantly and Calcium increased even if not significantly

Conclusions: In conclusion IXS and PCS can be considered as kidney function markers as well as have systemic toxic effects. Diabetes seems to increase the concentration of the two metabolites. The use of pre-probiotics should be started in the early stages of kidney failure and certainly for periods longer than four months. Pre-probiotics could aid in preventing renal osteodystrophy.

Introduction

The uremic syndrome is provoked by a progressive number of compounds that are normally excreted by kidneys in healthy individuals. At least 90 compounds, often called uremic toxins, like indoxylsulphate (IXS) and p-cresylsulphate (PCS), have

been found increased in subjects with ESRD creating great harm to biological systems [1]; these uremic toxins come from the intestinal bacterial fermentation [2] of the proteins. These solutes are considered not only biomarkers of renal function, but according to some authors they contribute to the development of kidney disease. The accumulation of these

compounds has a negative impact on many body functions, especially on the cardiovascular system, as recently proved by several authors who underline the association among serum PCS, cases of general mortality and cardiovascular mortality either in chronic kidney disease (CKD) or in its final stage [3-5]. In addition, in a study of Schepers et al., it has been shown that PCS stimulates the basic leukocyte activity with pro-inflammatory effects, inhibiting the activated leukocyte function and inducing endothelial disorder [6], a condition that could induce cardiovascular events. PCS, a phenol 108 Da MW, is a terminal product of protein catabolism, produced by intestinal bacteria that metabolize tyrosine and phenylalanine [7,8].

As regards IXS it is metabolized by the liver from the indol, which is produced by the intestinal flora as a metabolite of tryptophan. IXS causes an endothelial disorder of the uremia, promoting the proliferation of smooth muscle cells through the activation of growth factors derived from platelets and inducing a significant production of free radicals by endothelial cells. IXS appears to have a clinically important role in aortic stiffness and vascular calcification [9-11].

PCS and IXS both come from bacterial fermentation of the proteins in the large intestine: the colonic microbiota degrades tryptophan to indole. Therefore in renal failure conditions, the altered intestinal bacterial metabolism changes serum concentrations of IXS and PCS, so we wonder whether it is possible to induce a decrease of their serum concentrations. At this regard it was found that the intake of prebiotic inulin, enriched with oligofructose, could significantly reduce serum concentrations of PCS and IXS [12]; therefore clinical studies investigating the role of prebiotics and / or probiotics in CKD patients, would be useful in order to evaluate the possible positive impact on the evolution of chronic renal failure prevention. The aim of our study is to evaluate the short-term effects after an administration of pre / probiotics in CKD patients, regarding the production and then the serum concentrations of free IXS and PCS (i.e. non-protein bound fraction) and total IXS and PCS (i.e. sum of unbound and protein bound fraction).

Materials and Methods

In our study, 26 patients with CKD stage 2-5 associated with hypertension and / or diabetes mellitus type 2 (Table 1) were enrolled, and administered with 2 g/day dose of pre-probiotics for four months: mixed oligofructose (prebiotic component) + Lactobacillus acidophilus and Bifidobacterium longum (probiotic component). In all patients, at the beginning of the study, kidney function tests, glucose metabolism, PTH and blood uric acid were evaluated. Free and total PCS and

IXS were also measured. The two metabolites were analyzed in fresh or frozen serum sample (in fact the stability of the compounds allows the two conditions). The method involves the denaturation and precipitation of serum total proteins for the separation of the supernatant, on which the total PCS and IXS will be measured (sum of the protein bound fraction and the unbound fraction). The free fraction of these metabolites is achieved by centrifugal filtration in order to remove the binder's proteins. The dosage is performed by HPLC / MSMS using PCS-D4 as internal standard. At the same time 20 control subjects with normal renal function were considered in relation to the same parameters (Table 1).

After 4 months of daily intake of pre-probiotics, 19 patients were re-evaluated in relation to the same parameters. Statistical differences were studied using the Student-t paired and unpaired tests.

Results

The baseline values of free and total IXS and PCS of the 26 patients were significantly higher compared with the 20 normal subjects (Table 1). Serum concentrations of both metabolites importantly increased with reduced renal function and with the transition to the higher stage of CKD (Figure 1). The values of both the metabolites, free and total, in stage 2-3 CKD were significantly lower, although higher than normal subjects, in respect to stage 4-5 CKD in which increased by over 100% (Figure 1). Within the group of 26 patients, higher mean values of IXS and PCS in 12 diabetic subjects were highlighted, although not statistically significant (except for

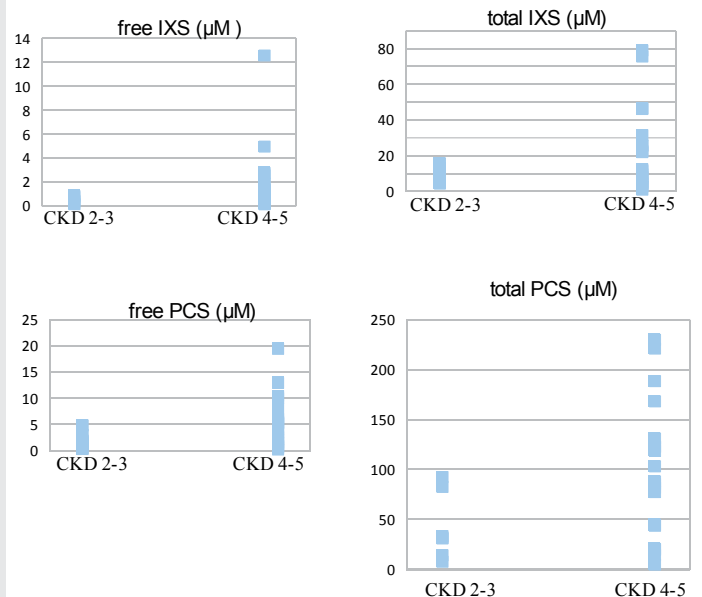


Figure 1: Free IXS, total IXS, free PCS and total PCS Trend depending on different CKD Stage.

Table 1: IXS and PCS Basal Values in 26 CKD 2-5 patients vs 20 controls.

IXS (free) µmol/l		IXS (total) µmol/l		PCS (free) µmol/l		PCS (total) µmol/l	
CKD 2-5	CONTROLS	CKD 2-5	CONTROLS	CKD 2-5	CONTROLS	CKD 2-5	CONTROLS
1,4 ± 2,51	0,1 ± 0,04	19,57 ± 21,02	3,18 ± 1,41	4,65 ± 4,56	0,31 ± 0,21	84,99 ± 64,63	12,9 ± 7,42
P<0,002		P<0,0008		P<0,00008		P<0,00005	

the free PCS that was significantly higher, $P < 0.02$), compared to 14 hypertensive non-diabetic patients (Table 2).

The 4-months re-evaluated data after the use of pre-probiotics in 19 patients (5 patients were out of the study for non-compliance of the processing and 2 patients for dialysis entrance) that completed the treatment protocol, showed increased concentrations of free and total IXS and PCS (Table 3). Considering renal function (7 patients in stage 2-3 CKD vs 12 patients in stage 4-5 CKD) the use of pre-probiotics increased the concentration of free and total PCS and IXS in all conditions, while remaining significantly higher in patients at

stage 4-5 rather than in the ones at the stage 2-3 (Tables 4,5). Considering the 9 diabetics patients and the 10 hypertensive non-diabetics patients, the use of pre-probiotics increased the free and total IXS and PCS serum concentrations, remaining significantly higher in diabetics rather than in hypertensive patients (Tables 6,7). In all periods, both baseline and after the uptake of pre-probiotics, the other measured parameters didn't change, except serum PTH that decreased significantly and Calcium increased even if none significantly (Table 8).

Discussion

Decades ago, nephrologists already thought that intestinal

Table 2: IXS and PCS Basal Values in 12 diabetic patients vs 14 hypertensive patients.

IXS (free) $\mu\text{mol/l}$		IXS(total) $\mu\text{mol/l}$		PCS (free) $\mu\text{mol/l}$		PCS(total) $\mu\text{mol/l}$	
DIABETICS	HYPERTENSIVE	DIABETICS	HYPERTENSIVE	DIABETICS	HYPERTENSIVE	DIABETICS	HYPERTENSIVE
2,32 \pm 3,51	0,77 \pm 0,72	22,35 \pm 20,5	17,19 \pm 21,56	6,95 \pm 5,57	2,66 \pm 2,15	107,23 \pm 64,4	65,94 \pm 60,63
ns		ns		P<0,02		ns	

Table 3: IXS and PCS Values in 19 CKD 2-5 patients after 4 months on probiotics therapy vs basal values.

IXS (free) $\mu\text{mol/l}$		IXS(total) $\mu\text{mol/l}$		PCS (free) $\mu\text{mol/l}$		PCS(total) $\mu\text{mol/l}$	
BASAL	POST	BASAL	POST	BASAL	POST	BASAL	POST
1,11 \pm 1,24	1,88 \pm 3,2	15,74 \pm 13,62	30,97 \pm 35,9	4,41 \pm 3,53	4,6 \pm 3,28	83,14 \pm 58,26	105,24 \pm 63,66
ns		P<0,05		ns		P<0,07	

Table 4: IXS and PCS basal Values in 7 CKD 2-3 patients vs 12 CKD 4-5 before 4 months of probiotics therapy.

IXS (free) $\mu\text{mol/l}$		IXS(total) $\mu\text{mol/l}$		PCS (free) $\mu\text{mol/l}$		PCS(total) $\mu\text{mol/l}$	
STAGE 2-3	STAGE 4-5	STAGE 2-3	STAGE 4-5	STAGE 2-3	STAGE 4-5	STAGE 2-3	STAGE 4-5
0,49 \pm 0,27	1,47 \pm 1,45	9,29 \pm 4,27	19,51 \pm 15,87	2,0 \pm 1,57	5,82 \pm 3,64	42,29 \pm 32,77	106,98 \pm 57,33
P<0,05		P<0,05		P<0,005		P<0,005	

Table 5: IXS and PCS Values in 7 CKD 2-3 patients vs 12 CKD 4-5 after 4 months of probiotics therapy.

IXS (free) $\mu\text{mol/l}$		IXS(total) $\mu\text{mol/l}$		PCS (free) $\mu\text{mol/l}$		PCS(total) $\mu\text{mol/l}$	
STAGE 2-3	STAGE 4-5	STAGE 2-3	STAGE 4-5	STAGE 2-3	STAGE 4-5	STAGE 2-3	STAGE 4-5
0,53 \pm 0,20	2,68 \pm 3,95	28,8 \pm 48,01	32,25 \pm 29,11	2,41 \pm 1,88	5,88 \pm 3,30	69,04 \pm 41,04	126,37 \pm 66,28
P< 0,09		ns		P<0,009		P< 0,03	

Table 6: Basal IXS and PCS Values after 4 months of probiotics therapy in 10 hypertensive patients.

IXS (free) $\mu\text{mol/l}$		IXS(total) $\mu\text{mol/l}$		PCS (free) $\mu\text{mol/l}$		PCS(total) $\mu\text{mol/l}$	
BASAL	POST	BASAL	POST	BASAL	POST	BASAL	POST
0,8 \pm 0,81	1,15 \pm 1,07	14,1 \pm 13,84	31,37 \pm 40,47	2,83 \pm 2,1	3,4 \pm 2,89	58,5 \pm 44,78	92,97 \pm 68,28
P <0,02		ns		ns		P <0,07	

Table 7: IXS and PCS Values and after 4 months of probiotics therapy in 9 diabetics patients.

IXS (free) $\mu\text{mol/l}$		IXS(total) $\mu\text{mol/l}$		PCS (free) $\mu\text{mol/l}$		PCS(total) $\mu\text{mol/l}$	
BASAL	POST	BASAL	POST	BASAL	POST	BASAL	POST
1,44 \pm 1,58	2,7 \pm 4,62	17,57 \pm 13,96	30,54 \pm 32,53	6,17 \pm 4,06	5,93 \pm 3,32	110,52 \pm 61,48	118,89 \pm 58,96
ns		ns		ns		ns	

Table 8: Renal function and serum values before and after 4 months of probiotic therapy in 19 patients.

	CKD-EPI ml/min	CREATININE mg/dl	AZOTEMIA mg/dl	URICEMIA mg/dl	PTH pg/ml	Ca mg/dl	P mg/dl	Hb gr/dl
BASAL	25,6 \pm 15,3	3 \pm 1,6	95,2 \pm 44	5,6 \pm 1,4	74 \pm 44	9,3 \pm 0,5	4 \pm 1	12,5 \pm 1,6
POST	25,7 \pm 17,2	3,4 \pm 2,5	110 \pm 56	6,1 \pm 1,7	46 \pm 25 P< 0,02	9,4 \pm 0,5	4,1 \pm 1,5	12,5 \pm 1,8

putrefaction products, in particular “ the indican”, were detectable in the blood of patients with “renal failure” and they played an important role in renal failure symptoms [13,14]. These studies showed that the concentration of indican a tryptophan derived from the oxidation product of indole, was higher in the blood of uremic patients rather than in the control subjects , and that several other aromatic compounds as phenols, cresols or aromatic oxyacids would produce strong oxidizing reactions up to trigger the formation of IXS and PCS [13,14]. Therefore our data seem to confirm what has already been reported by Wu et al., that IXS and PCS significantly increase with decreasing renal function and they can be regarded as valid markers for the progression of CKD [5]. This illustrates one of the fundamental problems we have to face during uremia studies. The uremic retention solutes move in the same direction, and when the glomerular filtration rate decodes the concentrations of uremic retention solutes, and most likely a number of unknown solutes, increase. In fact, Wu et al. observed in their studies a moderate correlation between IXS and PCS and estimated glomerular filtration rate (eGFR) and between concentrations of PCS and IXS [5,9]. The data of our study confirmed this theory, so we agree that from a statistical point of view IXS and PCS can be considered mostly markers of renal function as well as have systemic toxic effects.

The actual mechanism of insulin resistance in patients with CKD have not been clearly demonstrated, but a growing evidence suggests that the progressive increase of compounds, normally excreted by healthy kidneys, could play a key role in that mechanism. In a recent study, D'Apolito et al. proved that increased levels of urea in CKD patients may induce insulin resistance [14]. However, no study has already ruled out the role of protein-bound uremic toxins in the development of insulin resistance and metabolic disorders in CKD patients.

Koppe et al., supposed that the increase of the PCS concentrations associated with CKD may lead to insulin resistance and related metabolic disorders [12]. The increase of IXS and PCS concentration in diabetics patients (an aspect that we have demonstrated in our diabetic pts in respect to non-diabetics) could be caused by altered insulin or intestinal metabolism (preliminary studies on bacterial populations of the microbiota of diabetic subjects are currently in progress). Other studies seem to show that an increase of the two metabolites, in diabetic subjects, is the main cause of the damage of profibrotic kidney and widespread vascular tissue. Regarding to the use of pre-probiotics, some studies report positive effects on the reduced production of IXS and PCS. In fact an increase in the nutritional protein load, even in healthy individuals, results in an increase of their production and urinary excretion [3]. Therefore the concentration of serum IXS and PCS in uremic patients can be decreased by switching to a low-protein diet [6]. IXS and PCS are metabolites of tyrosine and phenylalanine, and they are converted to acid 4-hydroxyphenylacetic by intestinal bacteria, before being decarboxylated [7]. The main bacteria that contribute to the IXS and PCS rot are both aerobic (predominantly enterobacteria) and anaerobes (especially *Clostridium perfringens*) [8]. During the CKD, changes in the intestinal flora stimulate the overgrowth of specific bacteria

that are producers of IXS and PCS [8]. The administration of antibiotics reduces its urinary excretion, as a result of bacterial clearance that produces them [15, 16]. However, they can be reduced by orally administration of AST-120 absorbent (not sold in Italy), with a consequent serum decrease of IXS and PCS [18]. The gastric acids suppression, using omeprazole, promotes malabsorption of proteins and fermentation, resulting in an increase of their production [11]. Other environmental factors, such as the use of pre-probiotics may help to change the bacterial flora and thereby reduce the putrefaction which is the basis of the IXS and PCS production and absorption. Therefore, a diet with a small amount of animal protein [6], as well as an extra administration of *Lactobacillus* [16–18], decreases the IXS and PCS production. In our experience, an observation period of 4 months of daily intake of pre-probiotics associated with a low-protein diet (0.6–0.8 g of proteins per kg of body weight, based on the CKD stage), was not sufficient to prove the efficacy in all CKD stages; it is our opinion that their use should start and carried on for indefinite time, in the early stages of CKD (stages 2–3) and that it would be useless in later stages of CKD (stages 4–5). 19 patients after 4 months of pre-probiotic intake showed a significant reduction in PTH and a slight increase in serum total calcium despite therapy had remained unchanged since the beginning of the study. In this context the work of Campbell JM noted that bacteria of probiotic component, produce short chain fatty acids which decrease PTH followed by an increase in Calcium absorption via their solubilisation [19]. So in our opinion pre-probiotics could help the therapeutical strategy against osteodystrophy, during the various stages of CKD, in combination with vitamin D or other calcimimetics .

Conclusions

In conclusion IXS and PCS can be considered as kidney function markers as well as have systemic toxic effects. Diabetes seems to increase the concentration of the two metabolites. The use of pre-probiotics should be started in the early stages of kidney failure and certainly for periods longer than four months. Pre-probiotics could aid in preventing renal osteodystrophy.

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