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BRIEF REPORT

Probiotic dietary supplementation in patients with stage 3 and 4 chronic kidney disease: a 6-month pilot scale trial in Canada

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ABSTRACT

Aim: This was a pilot clinical trial to assess biochemical and clinical effects of an oral probiotic dietary supplement in chronic kidney disease (CKD) patients (stages 3 and 4).

Methods: A prospective, randomized, double-blind, crossover, placebo-controlled, 6-month trial of probiotic bacteria was conducted in 16 outpatients in Ontario, Canada. Primary endpoints included effect on hematologic, biochemical, and fecal variables, and on general well-being as assessed by quality of life (QOL). These outcomes were evaluated from biochemical parameters, mainly blood urea nitrogen (BUN), creatinine, uric acid, and C-reactive protein (CRP) as a general inflammatory marker. QOL was assessed on a subjective scale of 1 to 10 as the secondary parameter.

Trial registration: This pilot study forms part of registered trial NCT00760162.

Results: A total of 13 patients completed the study. Three patients dropped out: one was the receiver of a transplant. The second dropped out for unknown reasons and the third died of myocardial infarction (unrelated to probiotic bacteria or the protocol). Among the 13 patients who completed the trial, the mean change in BUN concentration during the probiotic treatment period (−2.93 mmol/L) differed significantly ($p = 0.002$) from the mean change in BUN concentration during the placebo period (4.52 mmol/L). In addition, the mean changes in uric acid concentration were moderate during the KB period (24.70 μmol/L) versus during the placebo period (50.62 μmol/L, $p = 0.050$), and the changes in serum creatinine concentration were insignificant. Neither gastrointestinal nor infectious complications were noted in any subject with improved QOL.

Conclusion: Orally administered probiotic bacteria selected to metabolize nitrogenous wastes may be tolerated for as long as 6 months. A major limitation of this trial is its small size that may have precluded detection of changes in other biochemical or hematologic parameters that would be evident in larger cohorts. Extension of the evaluation of this probiotic bacterial mixture will include a dose escalation trial in a similar prospective, placebo-controlled, and double-blind study site.

Introduction

Chronic kidney disease (CKD) progresses at varying rates, depending on etiology, to irreversible uremia. CKD was known to be a uniformly fatal disorder until the introduction of renal replacement therapies consisting of hemodialysis, peritoneal dialysis, and kidney transplantation permitted decades or longer of survival. Currently, however, the cost of renal replacement therapy, amounting to more than $80 000 annually in the United States, is prohibitive for most of the world limiting its broad application to industrialized so called 'rich' countries. In order to extend uremia therapy to less affluent countries, its cost must be sharply reduced. Determining whether a mixture of three specific strain combinations of naturally occurring beneficial microbes (known as probiotics) administered orally might metabolize nitrogenous wastes that accumulate as renal function declines has been the subject of investigation for over a decade by Kibow Biotech, Inc., USA.

Intestinal micro flora of animals and humans represent a complex ecosystem containing several species of both beneficial and harmful microorganisms. In recent years the concept of 'probiotics' has been the focus of attention by health professionals[1,2]. Probiotics are commonly defined as live microorganisms, which, when administered in adequate amounts, confer a benefit on the host[3,4]. As their safety and benefits are substantiated, it is reasonable to anticipate that probiotic bacteria will be incorporated into a growing number of clinical regimens[5].

Kibow Biotics*(KB), an oral probiotic formulation based on testing in rats[6–10] and miniature pigs[11–13], may serve as a potential dietary supplement to maintain a natural metabolic and physiological process in the kidney. The technology is based on the concept of 'Bowel for Kidney'*, in which bowel removal of nitrogenous wastes substitutes for the renal excretory function. Over the past 10 years, the potential utilization of probiotic bacteria as an adjunctive in induced animal and human applications has been evaluated. A combination of three bacterial strains (*Lactobacillus acidophilus* KB31, *Streptococcus thermophilus* KB27, and *Bifidobacterium longum* KB35) appears promising in four pre-clinical studies. The formulation tested in the present trial contains all three strains and may find potential application as a dietary supplement, decreasing concentrations of nitrogen containing metabolites and helping to maintain healthy kidney function[14].

Probiotics have potential dietary supplement uses (to help maintain healthy organ functions) and drug uses (to prevent or treat disease). This pilot study addressed only the potential dietary supplement use of KB for helping to maintain a healthy kidney function. After *in vitro* investigations, orally administered probiotic bacteria[15–30] were studied in 5/6th nephrectomized rats and minipigs. Further testing was carried out in veterinary practice in cats and dogs[31,32] with moderate-to-severe kidney failure. This testing documented the efficacy of the probiotic formulation in stabilizing natural metabolic processes and thus helping to maintain a healthy kidney function.

In addition to dietary supplement uses, probiotics may also be useful as drugs to prevent or treat diseases. A multisite clinical trial is now in progress to determine whether or not daily oral administration of a probiotic dietary supplement product formulation delays onset of and/or improves signs and symptoms of human CKD[33]. The hypothesis being tested is that a specifically formulated probiotic dietary supplement product comprised of defined and tested microbial strains may afford renoprotection and possibly alleviate the symptoms of uremic syndrome. An identical multisite clinical investigation with the same probiotic dietary supplement formulation is also in progress in Argentina, Canada, Mexico, Nigeria and the USA.

This pilot scale study was designed to examine the consequences of oral administration of probiotic bacteria (KB) in humans. Specifically, this involved: (a) assessing kidney function, (b) quantifying consequences of KB administration, (c) assessing safety, and (d) exploring the effects of the tested probiotic bacteria on the intestinal micro flora.

Methods

General regulatory overview

This pilot study (part of NCT00760162), which enrolled 16 patients, was approved by the Canadian Ethics Review Board (Optimum Clinical Research, Inc., 231 King St. E, Oshawa, ON, L1H 1C5, Canada) on February 28, 2007. It is an exploratory evaluation of oral ingestion of a proprietary probiotic product formulation intended as a dietary supplement formulation in the field of complementary and alternative medicine. The first author of this paper was responsible for interpreting the data (except the fecal analysis investigations) and writing the report. The fecal analysis data was analyzed, interpreted and compiled by Venkat Rao, PhD, University of Toronto, Ontario, Canada. The study was performed from July 2007 to August 2008.

*Kibow Biotics and 'Bowel for Kidney' are registered trademarks of Kibow Biotech, PA, USA

Inclusion and exclusion criteria

Patients were recruited between 7/23/07 and 2/20/08. Those patients who satisfied the following criteria were offered enrollment in the study: (1) age 18–75 years, (2) CKD stage 3 or 4, and (3) serum creatinine >2.5 mg/dL. CKD subjects were used in this study on the hypothesis that their abnormal gut flora would make it more likely to see a dietary supplement utility in a small number of subjects.

The following exclusion criteria were used: (1) pregnant or nursing women, (2) antibiotic treatment at the time of screening or within 14 days before screening, (3) refusal to sign the informed consent form, (4) active dependency on drugs or alcohol, (5) HIV/AIDS/liver disease, (6) any medical, psychiatric, debilitating disease/disorder or social condition that in the judgment of the investigator would interfere with or serve as a contraindication to adherence to the study protocol or ability to give informed consent or affect the overall prognosis of the patient, and (7) current anticoagulant therapy.

Study design

A pilot-scale, randomized, double-blind, placebo-controlled crossover clinical study was designed. Once the eligibility criteria had been met, the patients were randomized into two study arms: Group A and Group B.

Group A received the placebo; Group B received probiotic bacteria in the formulation, KB.

After 3 months, the crossover was made.

Group A received probiotic bacteria; Group B received the placebo.

This study design was chosen in an outpatient setting so that each patient himself/herself was considered as a nutritional control in both arms of the study, i.e. each patient acted as his or her own control (Figure 1).

Recruitment

After a potentially eligible patient was identified, according to the inclusion and exclusion criteria, informed consent was obtained and the patient was screened. The following assessments were conducted: medical history, documentation of disease/disorder, physical examination/clinical assessment, measurement of biochemical markers (serum creatinine, blood urea nitrogen), urinalysis and calculated urine protein-to-creatinine ratio, ammonia, ALT (alanine aminotransferase), CRP (C-reactive protein), ultrasonography KUB (kidneys, ureters, bladder), pregnancy test (if applicable), and HIV (human immune deficiency virus) test. The patient was also randomly assigned into one of the two study arms, dietary advice was given, and the study product/placebo was dispensed along with a patient diary card.

Treatment period

Patients were seen monthly during both 3-month treatment periods. Physical examination and complete laboratory testing were performed at each visit. (The following tests were included: blood biochemistry, hematology, liver function and urine protein to creatinine ratio, ALT, CRP, ammonia, adherence and quality of life assessment based on the patient diary card.) In addition, feces samples were collected at the beginning, the middle (3 months), and the end (6 months) of the study. Study product/placebo for the subsequent period was dispensed at each visit. No wash-out period was considered because of the cross-over design of this study. Any residual effect of either the treatment or placebo would have been negated since the data was evaluated from the 6-month minus the 3-month data monitoring.

Study product

KB is formulated with food-grade, Gram-positive bacteria. Each enteric-coated (for targeted ileo-cecal delivery) size 1 gel capsule contains a mix of *L. acidophilus* KB31, *B. longum* KB35, and *S. thermophilus* KB27, for a total of 1.5×10^{10} colony-forming units (CFU). Two capsules were administered three times daily, with meals (breakfast, lunch and dinner), for a daily dose of 9×10^{10} CFU. A normal healthy bowel contains

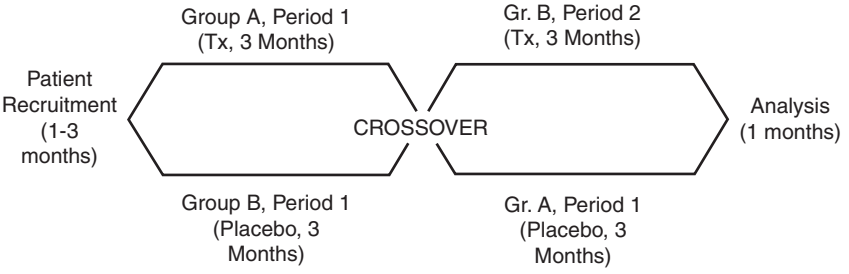

Figure 1. *Clinical study design*

1–2 kg of microbes and these are present in the amount of several hundred trillion CFU. Therefore, consumption of 90 billion CFU per day is clinically safe. The placebo was composed of wheat germ plus Psyllium husks. It was also matched in color, size and enteric coating identical to the interventional product. Randomization sequence was generated by alternative patient sequential methodology.

At the beginning of the first 3-month treatment period, patients were randomly and arbitrarily assigned to Group A or B and provided capsules containing either placebo or KB probiotic formulation (90 billion CFU/day, 15 billion/gel cap, 2 caps × 3/day). This was followed by crossover and the second 3-month treatment period.

Laboratory methods

Biochemistry and hematology

Complete blood counts, serum biochemical testing, and urine protein and creatinine (critical for creatinine is >650 μmol/L) measurements were performed by Gamma-Dynacare Inc. laboratory (Canada). The conversion of serum creatinine level to eGFR data was not considered important since this study was a short-term 6-month study. It was also too short a trial period to rely on or interpret the eGFR data.

Fecal analysis

Fecal analysis was performed at the microbial labs, Division of Nutrition, University of Toronto, Canada. Each patient was given a stool collection kit consisting of a collection bowl, a plastic toilet insert and plastic bags for fecal sample collection. Special syringes with tips removed for the purpose of fecal sampling, preweighed specimen vials containing appropriate media for microbiological enumeration, and styrofoam containers with dry ice were also provided. Immediately following defecation, subjects were instructed to use a syringe to transfer 1–2 g of fecal material into two separate vials, mix thoroughly, place them into the dry ice container, and later transport it to the laboratory for analysis. Subjects were trained in the process of fecal collection by a technician. At the time of analysis, fecal samples were thawed, mixed, and aliquots were taken for dilution. Measurements were made of fecal pH (using a pH meter) and microbial counts. Fecal samples were collected according to the prescribed protocol and analyzed for total aerobes (TAE), total anaerobes (TAN), *Bifidobacteria* (BIF), *Lactobacillus* (LAC), *Streptococcus* (STRP) and pH.

Statistical methods

Data are reported as mean ± SD. Differences were evaluated using analysis of variance and Student's *t*-test. Relationships between variables were assessed by means of linear regression analysis and a specific software program was used to determine significance. Results are considered significant for p-value ≤ 0.05 (95% confidence).

Results

Patients

Sixteen patients were enrolled in the study at the main participating site (Corporate Medical Center, Scarborough, Ontario, Canada), 13 of whom completed the study. Patient demographic information is listed in Table 1. Patients' age ranged from 40 to 70 years (mean 54 ± 8.8 years, $n = 13$), weight – from 53 to 130 kg (78.9 ± 22.7 kg, $n = 12$), height – from 157 to 184 cm (171 ± 8.8 cm, $n = 12$). Male subjects outnumbered female subjects by a factor of 9 to 4. Ethnicity was represented by eight Asians, three Caucasians, one Latino/Hispanic, and one African American. All but two subjects had hypertension, accompanied by one or more of the following comorbidities: hyperlipidemia, type 2 diabetes mellitus, IgA nephritis, or polycystic kidney disease (PKD). One patient had focal segmental glomerulonephritis along with hypertension. The two normotensive subjects had: (a) mesangial proliferative glomerulonephritis and (b) systemic lupus erythematosus with nephritis and hyperlipidemia. All patients were under treatment with three or more medications for the above conditions, as well as for hypocalcemia, hyperkalemia, hyperphosphatemia, anemia, proteinuria, acidosis, heartburn, anxiety, depression, and others.

Duration of treatment

Duration of treatment was 6 months in all patients who completed the study. Three subjects did not complete the study for the following reasons: one patient received a kidney transplant, one patient withdrew during the second treatment period for unknown reasons, and one patient died at his home due to a myocardial infarction (unrelated to the study intervention).

Laboratory testing

Biochemistry

While a host of biochemical parameters were measured, this study focused on the following: creatinine, uric acid, BUN, and C-reactive protein (CRP). Blood was

Table 1. *Patient demographics*

Patient ID	Age	Sex	Ethnicity	Wt, kg	Ht, cm	Primary disease	Study completion	Medications
001 R-H	45	M	White	93	184	Hypertension	Completed	Accupril, multivitamins, sodium bicarbonate, Palafer
002 PYC	57	M	Asian	58	168	Hypertension, IgA nephritis	Completed	Adalat XL, Atacand, Crestor, Eprex, iron gluconate, Mavik, Rocaltrol, salmon fish oil
003 HHL	55	M	Asian	72	174	Hypertension, hyperlipidemia,	Completed	–
004 SHC	51	M	Asian	53	170	Mesangial proliferative glomerulonephritis	Completed	Eprex, calcium carbonate, Cozaar, Crestor, Imuran, iron gluconate, prednisone
005 MJS	48	M	White	78	176	Hypertension, hyperlipidemia, cystic renal disease	Completed	Allopurinol, ASA (enteric coated), Atenolol, Avapro, calcium carbonate, Carbidopa/ Levoda, Colace, Crestor, Eprex, folic acid, isoniazid, Lasix, Lorazapam, Minoxidil, Norvasc, Palafer, Pantaloc, penicillin, sodium bicarbonate, Symbicort, Zestoret, Zoloft
006 SMC	50	F	Asian	65	157	Hypertension, hyperlipidemia, IgA nephropathy	Completed	Adalat XL, ASA (enteric coated), Atacand, calcium carbonate, Crestor, epo, Ezetrol, fish oil, folic acid, Palafer, Replavite
007 D-A	70	M	Asian	68	160	Hypertension, hyperlipidemia, diabetes type 2	Completed	Altace, ASA (enteric coated), Atenolol, Crestor, Diovan, folic acid, kayexalate cellulose, Novolin 30/70, Senocot, Tiazac XC
008 JRC	50	F	Asian	57	167	Hypertension, focal segmental glomerulonephritis	Completed	Avapro, calcium carbonate, Didrocal, Eprex, Palafer, Symbicort
009 S-X	45	M	Asian	97	174	Hypertension, IgA nephritis	Renal transplant. Withdrew	Adalat XL, Avapro, calcium carbonate, Replavite, Osteoforle
010 D-C	56	M	Asian			Hypertension, hyperlipidemia, PKD	Completed	ASA (enteric coated), Atenolol, Crestor, epo, folate, Minoxidil, Palafer, Plendil
011 IAM	61	M	Asian	75	175	Hypertension, PKD	Completed	Altace, calcium carbonate, calcitrol, Eprex, ferrous gluconate, Norvasc, sodium bicarbonate
012 Y-V	47	F	White	130	178	Hypertension, PKD	Completed	Atacand, calcium carbonate, Clomidine, Eprex, Norvasc, one alpha, Palafer, Replavite

(*continued*)

Table 1. Continued

Patient ID	Age	Sex	Ethnicity	Wt, kg	Ht, cm	Primary disease	Study completion	Medications
013 DJA	63	M	White	62	173	Hypertension, diabetes type 2	Died of MI at home (unrelated)	Altace, ASA (enteric coated), calcium carbonate, Crestor, Diovan, Eprex, Hytrin, Lasix, Metoprolol, Norvasc, one alpha, Palafer
014 N-M	40	F	Hispanic	72	157	Lupus nephritis, hyperlipidemia	Completed	Altace, Aliskiren, Alocrileye, Altarax, Avapro, calcium carbonate, Celestoderm/menthol Crestor, folic acid, Norvasc, Palafer, Replavite, sodium bicarbonate
015 D-M	43	M	Asian	79	171	Hypertension, congestive heart failure	Diarrhea, vomiting Withdrew after 1 visit	–
016 J-F	68	M	African Amer.	108	181	Hypertension, diabetes type 2	Completed	Avapro, Citalopram, Clonidine, Gluconorm, Ezetrol,Lasix, Levitra, Mavik, Metolazone, Minoxidil, Norvasc, Triferrex

drawn from each patient at every monthly visit. Subsequent to the study completion, relative changes in all four parameter levels were calculated separately for both treatment periods and for each patient. Biochemical data are presented in Table 2. Based on this cumulative data from all patients, relative changes based on the administered treatment – Kibow Biotics (KB) or placebo (PL) – were pooled and average relative changes for each parameter were calculated. Among the four parameters, there were significant changes observed in the level of BUN ($p = 0.002$) and uric acid ($p = 0.050$). The detailed results of these calculations are presented in Figure 2 and Tables 2, 3 and 4.

Fecal analysis

Our finding was that the total number of anaerobes especially *Bifidobacteria*, was much lower as compared to healthy individuals (10^{10} CFU/g). No significant changes were observed in the microbiological profiles between placebo and probiotic treatment groups after 90 days. However, levels of *Lactobacillus* were higher in the probiotic group compared to baseline and placebo groups. A trend towards a higher number of *Streptococcus* was also observed in both the placebo and probiotic groups as compared to the baseline.

Quality of life

Monthly overall QOL data during probiotic and placebo administration were evaluated on a score of 1 to 10. Since this was a pilot-scale study, we kept this questionnaire as simple as possible with the following easily understandable scale: 1,2 = very poor, 3,4 = poor, 5,6 = average, 7,8 = good and 9,10 = very good.

Adverse events

There was only one serious adverse event due to a myocardial infarction, which occurred at the patient's home. His medical history included type 2 diabetes mellitus (4–5 years), hypertension (3 years), smoker (47 years), diabetic retinopathy (1 years) and diabetic nephropathy (1.5 years), and anemia. Given this patient's history, the event of myocardial infarction is not related to the product as certified by the principal investigator, Dr Paul Tam. Minor events such as bloating or gas that were reported were of a temporary nature, lasting only a few days.

Discussion

This exploratory clinical trial's key finding is that a food-grade, Gram-positive bacteria mixture of

Table 2. *Clinical data, screening through visit 6*

Patient ID	Creatinine, μmol/L								Uric acid, μmol/L							
	Screen	Base	V1	V2	V3	V4	V5	V6	Screen	Base	V1	V2	V3	V4	V5	V6
001 R-H	370	scr	382	412	409	427	517	519	378		395	398	394	448	413	429
002 PYC	272	scr	279	275	281	309	262	270	362		410	511	524	545	523	490
003 HHL	338	304	280	309	335	307	315	290	569	485	485	454	479	400	464	519
004 SHC	267	303	276	292	363	283	288	261	748	788	418	768	891	801	768	676
005 MJS	495	scr	555	605	616	702	756		334		342	ND	307	358	392	
006 SMC	478	scr	519	416	443	479	467		538		596	588	634	633	649	
007 D-A	481	scr	485	448	514	569	644		555		536	512	493	506	494	
008 JRC	363	scr	415	382	381	355	377	397	583		477	526	592	ND	618	574
010 D-C	265	scr	270	255	266	268	264	322	615		627	607	583	669	593	676
011 IAM	391	scr	387	387	374	406	402		496		492	544	525	569	519	
012 Y-V	617	scr	553	597	741	741	825		361		372	343	423	395	292	
014 N-M	478	scr	470	487	516	656	696	559	495		485	506	472	ND	440	521
016 J-F	230	scr	222	213	219	223	211		546		561	577	632	607	633	
Initials	Blood urea nitrogen (BUN), mmol/L								CRP, C-reactive protein (CRP), mg/L							
001 R-H	16.6		16.5	16.6	17.5	14.7	22.4	21.9	1.1		1.1	4.5	1.3	1.1	0	0.8
002 PYC	15.3		15.8	20	16.8	19.7	14.6	16.2	0.2		<0.2	<0.2	0.2		0.3	
003 HHL	18.4	18.7	ND	14.6	17.3	16.6	ND	17.5	1	7.1	0.7	ND	ND	2.9	0.6	0.6
004 SHC	22.4	25.8	22.1	24.8	31	20	22.4	22.8	ND	0.3	2.9	ND	73.8	0.4	0.4	0.2
005 MJS	24.8		28.2	26.1	24.3	32.5	34.9				ND	ND	ND	0.5	10.3	
006 SMC	29		32.1	30.3	35.8	37	30.1		ND		1	0.8	ND	1.1	1.7	
007 D-A	25.5		24.4	20.3	23.6	24.1	29.4		ND		ND	1	0.8	0.9	15.2	
008 JRC	18.7		22	14.9	28.3	24.6	19.2	17.8	3.3		ND	ND	ND	ND	2.4	
010 D-C	12.1		11.8	12.7	16.2	15	13.7	18.3			0.2	0.3	21.1	0.7	0.4	ND
011 IAM	23.5		21.3	22.4	19.9	25.8	21.7		ND			6.9	10.3	4.4	ND	
012 Y-V	32.8		25.9	29.7	35.4	26.1	29.5		1.4		ND	0.8	2.2	2.2	2.5	
014 N-M	35		34.2	39	37.2	44.9	53.4	33.1			0.8	0.8	1.3	0.9	ND	2.1
016 J-F	18.9		16.8	22.1	19.4	18.8	ND		0.7		ND	0.6	0.8	1.4	1.3	

ND, not done; scr, baseline combined with screening

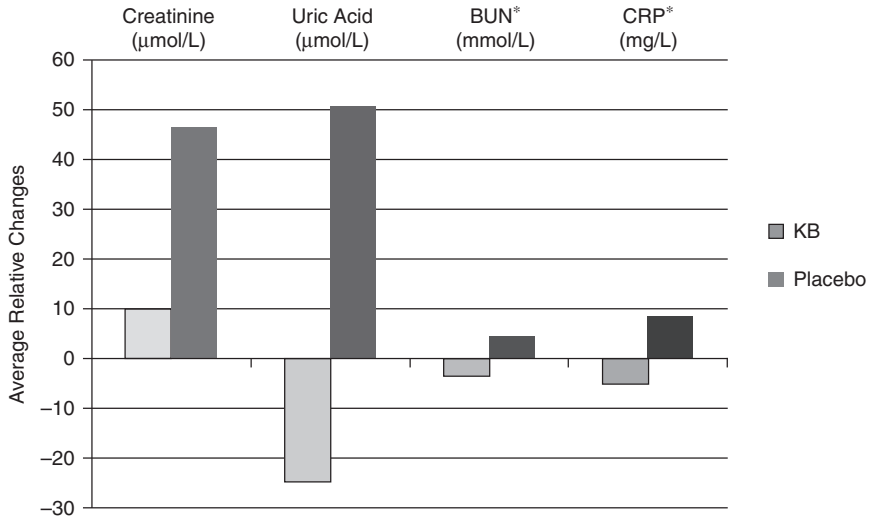

Figure 2. *Average relative changes in chosen biochemical parameters*

Table 3. *Changes in levels of biochemical uremic markers**

Patient ID	Treatment sequence	KB treatment period				PL treatment period			
		Creatinine, µmol/L	Uric acid, µmol/L	BUN, mmol/L	CRP, mg/L	Creatinine, µmol/L	Uric acid, µmol/L	BUN, mmol/L	CRP, mg/L
001 R-H	KB – PL	39	16	0.9	0.2	110	35	4.4	–0.5
002 PYC	PL – KB	–11	–34	–0.6	0.1	9	162	1.5	0
003 HHL	KB – PL	–3	–90	–1.1	–0.3	–45	40	0.2	–2.3
004 SHC	PL – KB	–102	–215	–8.2	–73.6†	96	143	8.6	73.5**
005 MJS	KB – PL	121	–27	–0.5	0	140	85	10.6	9.8
006 SMC	PL – KB	24	15	–5.7	0.6	–35	96	6.8	–0.2
007 D-A	KB – PL	33	–62	–1.9	–0.2	130	1	5.8	14.4
008 JRC	PL – KB	16	–18	–10.5	–0.9	18	9	9.6	0
010 D-C	PL – KB	56	93	2.1	0.1	1	–32	4.1	20.9
011 IAM	KB – PL	–17	29	–3.6	3.4	28	–6	1.8	–5.9
012 Y-V	PL – KB	84	–103	3.4	0.3	124	62	2.6	0.8
014 N-M	PL – KB	–97	49	–11.8	1.2	38	–23	2.2	0.5
016 J-F	PL – KB	–12	26	–0.6	–0.1	–11	86	0.5	0.1
Average changes		10.1	–24.7	–2.93	–5.32	46.4	50.6	4.52	8.55

*The data in this table is presented grouped by treatment, not according to treatment sequence, to demonstrate how the average relative values used in Figure 2 were obtained. For the first treatment period (KB or PL), data were obtained by subtracting values at screening from values at visit 3. For the second treatment period (KB or PL), data were obtained by subtracting values at visit 3 from values at the last visit (visits 5 or 6)

†This patient suffered from an acute infection during the period of the study, which explains the abnormally high level of CRP

BUN, Blood urea nitrogen; CRP, C-reactive protein; KB, Kibow Biotics, PL, placebo

probiotic product formulation (previously found to be beneficial to rodents and miniature pigs with renal failure) was well-tolerated when administered orally in two 3-month periods in a prospective double-blind placebo-controlled cross-over study (Figure 1). Values for the first treatment period data were obtained by subtracting values at initial screening from values at 3 months. Second treatment (after switching bacteria and placebo) values were derived by subtracting values at 3 months from values at 5 or 6 months.

Our primary endpoint was based on changes in blood chemistry (BUN, uric acid, serum creatinine, CRP and fecal analysis). Secondary analysis was measured with a simple QOL diary maintained by the patients on a subjective scale of 1 to 10. The data on weight, BMI, blood pressure, ammonia, calcium, phosphate and ALT levels were measured and recorded during the patient's monthly visits to the clinic, but they are not detailed herein because of very few changes were observed. Hence, they were not taken into consideration as our

Table 4. *Table of paired-sample test*

Paired differences								
Biochemical parameters	Mean	SD	SE mean	95% CI of the difference		T	DF	*p*-value 2-tailed significance
				Lower	Upper			
BUN	7.44615	6.79198	1.88376	3.34180	11.55051	3.953	12	0.002
Creatinine	36.30769	74.94596	20.78627	−8.98170	81.59708	1.747	12	0.106
Uric acid	75.30769	124.75869	34.60183	−0.08323	150.69861	2.176	12	0.050
CRP	13.86923	40.79711	11.31508	−10.78421	38.52268	1.226	12	0.244

BUN, Blood urea nitrogen; CRP, C-reactive protein; T, test for paired samples; DF, degrees of freedom

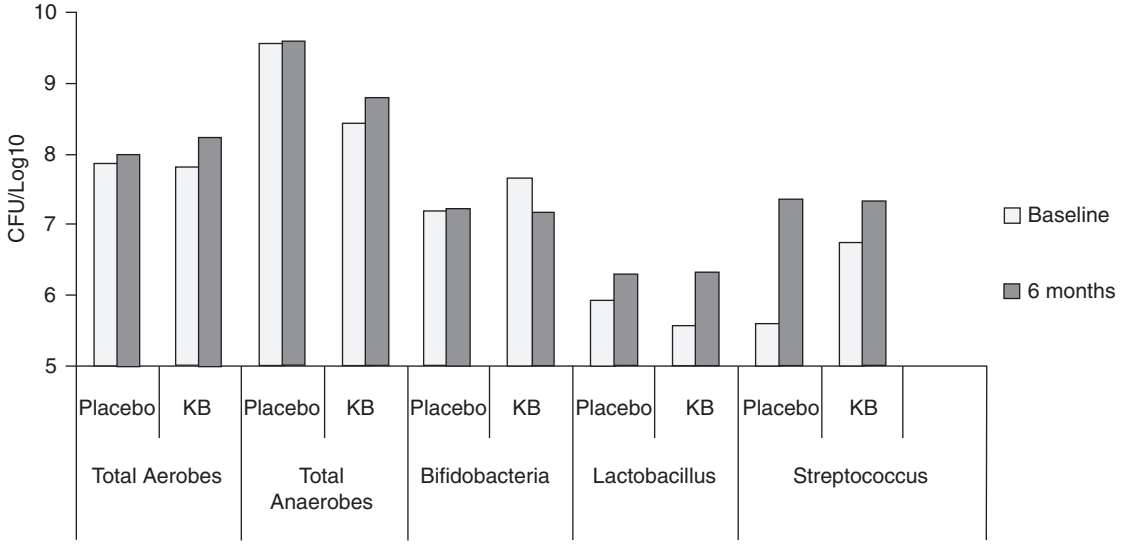

Figure 3. *Fecal microbial profiles*

primary or secondary markers for this pilot scale study, which is a limitation of this report.

Reductions in creatinine, urea, and uric could be influenced by loss of appetite during bacteriotherapy which could be a limiting factor in this study. However, this specific aspect or lack of appetite was not reported by any of the study patients in their daily QOL data. All three biochemical parameters followed a similar pattern evincing stability or decline during probiotic bacterial administration compared with stability or increase during administration of placebo (Tables 2 and 3). Mean biochemical group values for all subjects either decreased or remained stable (Figures 2 and 3) during probiotic bacterial administration, but either remained relatively stable or significantly increased during placebo administration. Assessing each patient's response when serving as his or her own control, it was noted that when marker levels did not increase during administration of probiotic bacteria, they increased during the placebo period. Similarly, when levels remained relatively stable during the placebo period, they invariably decreased during the administration of probiotic bacteria. As shown in Table 4, statistical analysis of the four variables monitored indicates that only BUN level changes were significant ($p = 0.002$). In addition, a moderate decrease in uric acid was also noted ($p = 0.05$).

However, there was no significant change observed in serum creatinine, which is considered a key marker for kidney function. The probiotic bacteria dose, estimated as 90 billion CFU per day in the current study, was extrapolated from an uncontrolled small trial in cats[34]. Clearly, both increased number of study subjects and an increased dose of probiotic bacterial product formulation to be administered per subject per day are modifications in protocol under consideration for subsequent studies.

All subjects were asked to maintain a symptoms diary during the study and record any unusual observations including bowl movements. None of the subjects

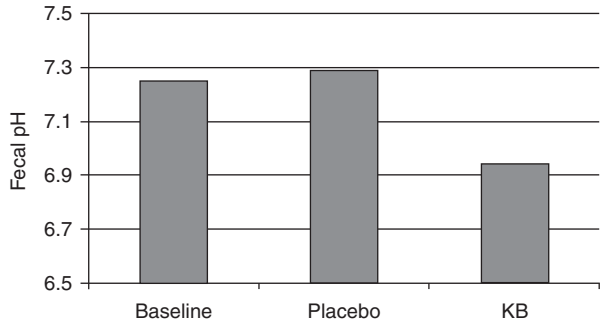

Figure 4. *Observed fecal pH values*

indicated experiencing symptoms of diarrhea. Observed fecal levels of *Bifidobacteria* were surprisingly low as depicted in Figure 3. Possible explanations for the low levels include: (a) insufficient dose in administered probiotic formulation, (b) instability of the selected strain of *Bifidobacteria*, or (c) incorrect handling of fecal samples to ensure sustained anaerobic conditions. Further studies have been initiated to discern the reason for the low levels of *Bifidobacteria* that reflect total fecal anaerobes. By contrast, the increased stool content of both *Lactobacillus* and *Streptococcus* at 90 days may reflect the intra-gut conversion of urea to ammonia, a source of nitrogen for multiple metabolic purposes, including additional microbial growth. Administering probiotic bacteria by reducing the 'ammonia burden', might substitute for missing renal excretion of ammonia in renal failure. Fecal pH of the probiotic bacteria cohort (pH = 6.94) was significantly lower than the placebo cohort (pH = 7.29) with a p-value of >95% (Figure 4). An alternative explanation for the lower stool pH in the probiotic bacteria cohort might be the administration of *Lactobacillus*, a species known to generate an acidic environment due to production or generation of lactic acid.

None of the patients withdrew from the trial because of objection to or adverse reaction to being fed bacterial microorganisms (probiotics). While multiple probiotic products are now marketed, it was encouraging to appreciate that patients with substantive chronic kidney disease were willing to participate in a study protocol that represented an initial human trial with an unknown outcome. In this context, each subject's self-assessment of quality of life (QOL) afforded insight into how ingestion of living bacteria might alter day-to-day behavior. Monthly overall QOL data during probiotic bacteria and placebo administration were evaluated on a score of 1 to 10 (1,2 = very poor; 3,4 = poor; 5,6 = moderate; 7,8 = good; 9,10 = very good). While overall QOL improved in all subjects during probiotic treatment from a mean of 6.68 to a mean of 7.77 ($p < 0.05$), the main inference is that QOL did not deteriorate when probiotic bacteria were administered. In the future trials, we intend to use a SF36 questionnaire, which will be a more appropriate tool for assessing QOL.

Proper nutritional assessment was not carried out as this was a small pilot scale study with limited resources. Hence, we designed the study with the cross-over design after 3 months so that the patient himself/herself will be a control. This may be construed as a weakness of this study. Besides the above discussions, the main limitations of the study are the small size of the sample group, non-adherence to SF36 for QOL, and absence of statistical positive change in serum creatinine, which is generally linked to eGFR reflecting kidney function characteristics. Whether the benefits noted in azotemic sub-totally nephrectomized rodents and miniature pigs will translate into benefit for human kidney failure continues as an unanswered question. Derivative, larger scale multicenter trials of probiotic bacteria in expanded dosage appear appropriate and highly recommended for future studies.

However, the strength of this study lies in its documentation that a small group of patients with chronic kidney disease ($n = 13$) completed a 6-month trial of probiotic bacteria with some indication of benefit in terms of significant reduction in BUN and a moderate reduction of uric acid. In addition, the absence of adverse reactions and improved quality of life were also outcomes from this study.

Conclusion

This study found that oral ingestion of a probiotic bacterial regimen in patients with CKD was well-tolerated, with decreases in BUN and uric acid, possibly contributing to an improved QOL. Full-scale clinical trials to test the potential applications for gut-based probiotic administration, including dose escalation studies, should be initiated to determine whether or not the addition of probiotics helps to stabilize natural metabolic processes. A larger clinical trial including dose escalation studies is planned.

Transparency

Declaration of funding

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Declaration of financial/other relationships

N.R. has disclosed that he is the Senior Vice-President of research and development at Kibow Biotech, Inc. P.R. has

disclosed that she is the Vice-President for clinical and regulatory affairs, Kibow Biotech, Inc. N.R. and P.R. have both disclosed that they hold a substantial combined business interest in Kibow Biotech, Inc. R.D. has disclosed that he is an employee of Kibow Biotech, Inc. E.A.F. has disclosed that he serves, without compensation, as Chair of Kibow Biotech's Scientific Advisory Board, and that his Renal Division currently receives research funding for a clinical trial of Kibow Probiotics. P.T. and V.R. have disclosed that they have not received any compensation from Kibow Biotech for conducting this pilot-scale clinical study.

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