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ORIGINAL RESEARCH

Pilot Study of Probiotic Dietary Supplementation for Promoting Healthy Kidney Function in Patients with Chronic Kidney Disease

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ABSTRACT

Introduction: Uremic syndrome consists of nitrogenous waste retention, deficiency in kidney-derived hormones, and reduced acid excretion, and, if untreated, may progress to coma and eventual death. Previous experience suggests that oral administration of a probiotic formulation of selected microbial strains may extend renoprotection via intraintestinal extraction of toxic waste solutes in patients with chronic kidney disease (CKD) stages 3 and 4. This report presents preliminary data from a pilot study. ***Methods:*** This was a 6-month prospective, randomized, double-blind, placebo-controlled crossover trial of a probiotic bacterial formulation conducted in four countries, at five institutions, on 46 outpatients with CKD stages 3 and 4: USA (n=10), Canada (n=13), Nigeria (n=15), and Argentina (n=8). Outcomes were compared using biochemical parameters: blood urea nitrogen (BUN), serum creatinine, and uric acid. General well-being was assessed as a secondary parameter by a quality of life (QOL) questionnaire on a subjective scale of 1-10. ***Results:*** Oral ingestion of probiotics (90 billion colony forming units [CFUs]/day) was well tolerated and safe during the entire trial period at all sites. BUN levels decreased in 29 patients (63%, P<0.05), creatinine levels decreased in 20 patients (43%, no statistical significance), and uric acid levels decreased in 15 patients (33%, no statistical significance). Almost all subjects expressed a perceived substantial overall improvement in QOL (86%, P<0.05). ***Conclusion:*** The main outcomes of this preliminary trial include a significant reduction

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of BUN, enhanced well-being, and absence of serious adverse effects, thus supporting the use of the chosen probiotic formulation for bowel-based toxic solute extraction. QOL and BUN levels showed statistically significant differences in outcome ($P<0.05$) between placebo and probiotic treatment periods at all four sites (46 patients). A major limitation of this trial is the small sample size and related inconsistencies.

Keywords: chronic kidney disease; disease progression; healthy kidney; probiotics; renoprotection; uremic syndrome

INTRODUCTION

Probiotics are defined by the Food and Agriculture Organization and World Health Organization in 2002 as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[1] Probiotics are increasingly appearing in functional (health-promoting) foods, beverages, supplements, and complementary medicine. Probiotics and probiotic foods have recently become popular in the United States, even though such products have been marketed for decades in Europe and Asia. Probiotic microbes are common in dairy foods such as yogurt, kefir, cheese, and other fermented foods and have been a focus of expanding medical investigation. Although widely used for decades in food and alcoholic fermentations, only fairly recently have these microbes undergone scientific scrutiny for their purported health benefits.[2] The expansion of our awareness and use of probiotics, however, has raced ahead of the scientific basis for their application.

Claims for beneficial effects of probiotics that are linked to research reports include: improving gastrointestinal tract health, enhancing the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing the symptoms of lactose intolerance, decreasing the prevalence of allergy in susceptible individuals, and reducing risks of certain cancers.[3,4] The mechanisms by which probiotics exert their effects are largely unknown, but may involve modifying gut pH, antagonizing pathogens through production of antibacterial compounds, competitive exclusions of pathogens at the binding and receptor sites, competing for available nutrients, and binding of deleterious mutagens and carcinogens.[5]

Over the past 12 years, Kibow Biotech Inc. (Newtown Square, PA, USA) has continuously explored the potential utilization of oral sorbents and nonpathogenic probiotics as complementary medicine in chronic kidney disease (CKD). Although proof of clinical efficacy of the tangible benefits of treatment with probiotics is mounting, their prescription remains underexplored commercially. Probiotics are not yet part of the clinical arsenal for prevention and treatment of disease or maintenance of health. Thus, we have patented our proprietary product formulation known as Kibow Biotics® (KB) that is specifically targeted to metabolize and extract uremic toxins as a component of our company's "gut-based" platform technology.

Kidney disease is ranked fourth among the major diseases in the United States, afflicting over 20 million people and growing at 8% annually.[6] Worldwide, the number of patients with CKD is rising and it is now being recognized as a major public health concern that is threatening to reach epidemic proportions over the next decade.[7] Existing worldwide statistical data on the incidence and prevalence of kidney disease and kidney failure, the resulting mortality, the high cost of treatment, and associated socioeconomic and political consequences present a compelling and urgent need for an effective alternative

adjunct treatment modality to be available to the global kidney patient population.

Uremic syndrome is said to consist of nitrogenous waste retention, deficiency in kidney-derived hormones such as erythropoietin and vitamin D (anemia and renal osteodystrophy), and reduced acid excretion (acidosis).[8] Untreated uremia may progress to coma and eventual death. Previous in-vitro and in-vivo investigations undertaken by Kibow Biotech in recent years suggest that oral administration of a probiotic formulation comprised of selected microbial strains may extend renoprotection via intraintestinal extraction of toxic solutes in patients with CKD stages 3 and 4.[9] This report presents some preliminary data from a pilot study involving 46 patients at five sites in four countries: Argentina, Canada, Nigeria, and USA (two sites).

The human intestinal microflora is complex with total counts of 10^{11}-10^{12} colony-forming units (CFU) per gram of stool.[10]Among this vast number of organisms are at least 400 species of anaerobes and many facultative organisms, within which are several species of *Lactobacilli* and *Bifidobacterium*.[11] Intestinal microflora comprises a highly active society of organisms, possessing a diverse complex of enzymes that perform extremely varied functions, both beneficial and detrimental. The delicate yet critical balance is maintained among this enormous bacterial population that plays an important role in maintaining not only intestinal health, but also the overall health of the host. Up to 80% of the body's immune system is localized in the gastrointestinal tract, indicating enormous chemical activity occurring in the intestine.[12] The bowel proffers unique opportunity to: (1) modulate immune responsiveness; (2) prevent or treat diseases arising in this organ; and (3) remove or modify uremic toxins in a therapeutic regimen. In this regard, uremic toxins constitute over 100 different metabolites, most of which diffuse into the bowel as a result of the kidney's inability to filter these waste metabolites. The mean distribution of some important uremic solutes diffusing into the bowel are shown in Table 1.[13]

Probiotics are viable organisms and supportive substances that improve intestinal microbial balance, such as *Lactobacillus acidophilus* and bioactive proteins.[14] These and other bioactive proteins have been shown to possess many biological activities (eg, insulin, whey protein, and various enzymes). Empirical evidence accumulated over many years links the use of fermented dairy products such as yogurt and milk to the promotion and maintenance of poorly defined intestinal health. The ability of *L. acidophilus* to help prevent pathogenic bacteria from proliferating and healthy bacteria from becoming toxic is well documented.[15-20] When the proper strain is chosen, it may help to maintain a population equilibrium, or balance between the different forms of microorganisms curtailing their potential overgrowth and pathogenicity.[21-25] *Bifidobacterium* is another probiotic organism that occurs naturally in the human intestine, with *Bifidobacterium infantis* being the first flora to colonize the intestines of breast-fed newborns. Research studies have documented several beneficial effects of bifidobacteria when given to infants, such as its effectiveness against a specific strain of

Table 1. Mean distribution of some uremic solutes (mg/100 mL).[13]

Solute	Gastric	Bile	Intestinal
Urea	75	107	92
Phenols	00	87	218
Creatinine	33	34	35
Uric acid	42	32	32
Guanidines	00	26	35
Indican	00	32	58

enteropathogenic *Escherichia coli*,[26] prevention of enteric infections,[27] and decreasing the growth of *Candida albicans*.[28,29] It is believed that *Lactobacillus* and *Bifidobacterium*[30] species help maintain the proper balance between the different forms of microorganisms in the intestine. They produce organic acids that may reduce pH in microenvironments of the gastrointestinal tract, and thereby inhibit acid-sensitive bacteria, including enteric pathogenic species. Lactobacilli, which frequently are more acid-tolerant than other organisms, produce lactic acid and hydrogen peroxide, and some species possibly acetic and benzoic acids.[31] Acids produced by bifidobacteria include short-chain fatty acids such as acetic, propionic, and butyric acids, as well as lactic and formic acids.[32,33] At optimal pH values they exert several inhibitory influences on bacterial cell growth.[34,35] The most abundant short-chain fatty acid produced by bifidobacteria is acetic acid, which exerts a wide range of antimicrobial activity against yeasts and molds as well as other pathogenic bacteria.

In addition to lactic and other acids, lactobacilli have the capacity to secrete numerous metabolites or endotoxins that kill pathogenic bacteria.[36-40] A variety of antibacterial or anti-yeast substances have been isolated such as lactocidin, lactobicillin, lactobreven, and acidolin.[33,34,36,40] Some benefit may be obtained through the administration of strains known to produce these agents as a part of their life cycle.[40]

Bacterial species that have traditionally been regarded as safe have been used in probiotics; the main strains employed include lactic acid bacteria (LAB) and bifidobacteria that inhabit the intestinal tracts of humans and animals. LAB in fermenting food have a long history of safe use. LAB such as *Lactobacillus* and *Enterococcus* are consumed daily. *L. acidophilus* has been safely used for more than 70 years. Some strains of *Streptococcus* and *Enterococcus* show the properties of LAB. *L. acidophilus* NCFMT^{M} strain (Danisco Inc., Madison, WI, USA) has been employed for eradication of small bowel bacterial overgrowth in dialysis patients.[41] Likewise, several strains of *Bifidobacterium* are extensively prescribed for patients with kidney failure in Japan (mainly targeted for removal of phenol, indole, and related aromatic metabolic uremic toxins)[42] and many such over-the-counter products are available in several countries as components of infant formulas and dietary supplement products. There is indeed a growing consensus on the beneficial effects of bifidobacteria in human health.

Streptococcus thermophilus (a high urease or urease utilizing microbe) is mainly present in fermented foods, particularly various yogurts and its derivative products. Many investigators have studied the therapeutic and preventive effects of yogurt and LAB, which are commonly used in yogurt production, on diseases such as cancer, infection, gastrointestinal disorders, and asthma.[43] Because the immune system is an important contributor to all of these diseases, an immunostimulatory effect of yogurt has been proposed and investigated by using mainly animal models and, occasionally, human subjects. Although the results of these studies, in general, support the notion that yogurt has immunostimulatory effects, problems with study design, lack of appropriate controls, inappropriate route of administration, sole use of in vitro indicators of the immune response, and short duration of most of the studies limit the interpretation of the results and the conclusions drawn from them. Nevertheless, these studies provide a strong rationale for the hypothesis that increased yogurt consumption, particularly in

immunocompromised populations such as the elderly, may enhance the immune response, which would in turn increase resistance to immune-related diseases. This hypothesis, however, needs to be substantiated by well-designed, randomized, double-blind, placebo-controlled human studies of an adequate duration in which several in-vivo and in-vitro indexes of peripheral and gut-associated immune responses are tested.

We hypothesized that oral administration of a probiotic formulation of selected microbial strains will have a stabilizing and beneficial effect on the quality of life (QOL) and may extend renoprotection via intraintestinal extraction of toxic solutes in patients with CKD stages 3 and 4. This report presents preliminary data from a pilot study.

METHODS

This was a 6-month prospective, randomized, double-blind, placebo-controlled, crossover trial of probiotic bacteria conducted in four countries, at five institutions, on 46 outpatients with CKD stages 3 and 4: USA (*n*=10), Canada (*n*=13), Nigeria (*n*=15), and Argentina (*n*=8). Hospital Juarez in Mexico City was also an institutional review board (IRB)-approved site; however, due to problems relating to the import of the study product, the study could not be carried out at this site. Primary measures included hematological, biochemical, and fecal (only at the Canadian site) variables. Outcomes were compared using biochemical parameters: blood urea nitrogen (BUN), serum creatinine, and uric acid. General well-being was assessed as a secondary parameter by a customized QOL questionnaire on a subjective scale of 1-10, rather than the generally used SF36 form, which was not used due to resource and time constraints.

General Regulatory Overview

This pilot study (NCT00760162) was approved by the Canadian Ethics Review Board (Optimum Clinical Research, Inc., Oshawa, ON, Canada) on February 28, 2007. Likewise, IRB approvals were obtained through the respective hospitals for the other study sites. It is an exploratory evaluation of an orally ingested proprietary probiotic formulation intended as a dietary supplement in the field of complementary and alternative medicine. Natarajan Ranganathan was responsible for interpreting the data (except the fecal analysis investigations) and writing the report. The fecal analysis data were analyzed, interpreted, and compiled by Venkat Rao, PhD (University of Toronto, Ontario, Canada). The study was performed from July 2007 to August 2009.

Inclusion and Exclusion Criteria

Patients were recruited between July 2007 and January 2009. 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 based 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.

1. Group A received the placebo; Group B received probiotic bacteria in the formulation, KB.
2. After 3 months, the crossover was made.
3. Group A then received probiotic bacteria; Group B then received the placebo.

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

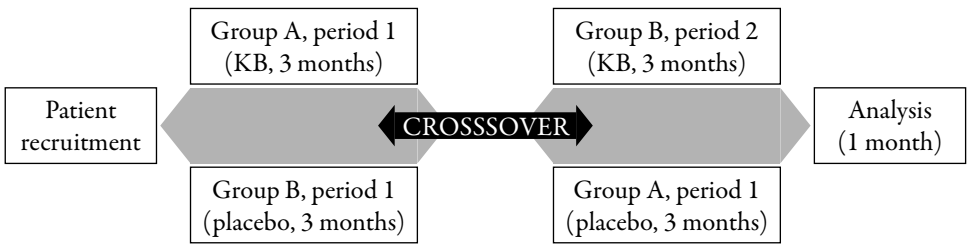
Recruitment

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, BUN), urinalysis and calculated urine protein-to-creatinine ratio, ammonia, alanine aminotransferase, C-reactive protein, ultrasonography of kidneys, ureters, bladder, pregnancy test (if applicable), and HIV test. The patient was also randomly assigned to begin in one of the two study arms (A or B), dietary advice was given, and the study product/placebo was dispensed along with a patient diary card.

Treatment Period

Patients were periodically examined every month at both 3-month 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, alanine aminotransferase, C-reactive protein, ammonia, adherence, and QOL assessment based on the patient diary card. In the case of Canada, fecal samples were also collected at the beginning, the middle-crossover (3 months), and the end (6 months) of the study.[9] Study product/placebo for the subsequent period was dispensed at each visit. No washout period was considered because of the crossover design of this study. Any residual effect of either the treatment or placebo would have been negated as the data were evaluated from the difference between third- and sixth-

Figure 1. Clinical study design. KB=Kibow Biotics.



month data monitoring with placebo against KB product interventional time.

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* KB27, *B. longum* KB31, and *S. thermophilus* KB19, for a total of 1.5×10^{10} 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 1-2 kg of microbes and these are present as several hundred trillion CFU. Therefore, consumption of 90 billion CFU per day is clinically safe and can be compared with oral consumption of 400-500 mg of common active pharmaceutical-like ingredients available as over-the-counter products. The placebo was composed of wheat germ plus psyllium husks. It was also matched in color, size, visual look, and enteric coating identical to the interventional product.

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 exclusively in Brampton, Ontario, Canada. At all other sites, blood analysis and biochemical testing were performed at their respective hospital's clinical lab. Hence, this certainly creates comparative variation in data analysis, as the increase or decrease of the biochemical parameters is dependent on the coefficient of variation (CV) of each lab methodology at each respective study site. No attempts were made to normalize or standardize the accurate performance of the study among the various four sites. The conversion of serum creatinine level to estimated glomerular filtration rate data was not considered important, as this study was a short-term 6-month study. It was also too short of a trial period to rely or interpret the estimated glomerular filtration rate data.

Fecal Analysis

Fecal analysis (only done at the Canadian site) was performed at the microbial labs, Division of Nutrition, University of Toronto, Canada. All of the enrolled patients voluntarily consented for collecting their own fecal specimen for analysis. 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. Fecal collection analysis was done by all 13 patients at the end of the second-, fourth-, and sixth-month time periods. 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, total anaerobes, bifidobacteria, *Lactobacillus*, *Streptococcus*, and pH.

Quality of Life

Patients were asked to rate their QOL on a scale of 1-10, which was coded as follows: 1, 2=very poor; 3, 4=poor; 5, 6=average; 7, 8=good; and 9, 10=very good.

Statistical Methods

Differences in average levels of creatinine, uric acid, and BUN between the placebo and the treatment periods were evaluated using analysis of variance and Student *t*-test. Differences in average change of QOL ratings between the placebo and the treatment periods were also evaluated. SPSS v.17.0 software was used to perform the tests and determine significance. Results were considered significant for $P \leq 0.05$ (95% CI).

RESULTS

Oral ingestion of probiotics (90 billion CFUs/day) was well tolerated and safe during the entire trial period at all sites. Of the 62 subjects enrolled (all four sites combined), 46 completed the study, with the rest being lost to follow-up (74% completion rate). A summary of patient demographic data and the average levels of the primary biochemical parameters by study period are presented for each study site in Table 2. Across all four sites, patients' age ranged between 21 and 76 (median age 57 years), males (31) outnumbered females (15), and 19 patients were diabetic. Other primary diseases included hypertension, polycystic kidney disease, and glomerulonephritis, as well as some cases of unknown etiology.

While a host of biochemical parameters were measured, this study focused primarily on creatinine, uric acid, and BUN. Blood was drawn from each patient at every monthly visit. In comparison with the placebo period, BUN levels were lower during the KB treatment period in 29 patients (63%, $P<0.05$), creatinine levels were lower in 20 patients (43%, no statistical significance), and uric acid levels

Table 2. Cumulative average levels by treatment period at all four sites (biochemical parameters expressed in μmol/L).

	Average age, years	Age group	Sex		D/ND		Creatinine (range)			Uric acid (range)			BUN (range)		
			M	F	D	ND	KB	PL	KB-PL	KB	PL	KB-PL	KB	PL	KB-PL
USA, *n*=10	64.3	40-76	7	3	7	3	278.9 (186 to 357)	284.0 (186 to 357)	47.2 (−111 to 49)	508.3 (323 to 672)	510.9 (351 to 639)	90.3 (−214 to 89)	18.7 (10.5 to 32.3)	19.3 (9.5 to 32.3)	2.3 (−4.2 to 3.2)
Canada, *n*=13	53.7	40-70	9	4	2	11	422.6 (217 to 783)	428.8 (218 to 729)	85.9 (−137 to 153)	524.8 (325 to 748)	499.6 (375 to 692)	47.8 (−35 to 140)	23.1 (15.7 to 43.8)	25.2 (14.5 to 38.1)	3.6 (−8.5 to 5.7
Argentina, *n*=8	57.5	21-74	6	2	4	4	312.0 (254 to 461)	314.4 (236 to 439)	20.7 (−35 to 22)	465.9 (389 to 593)	457.9 (335 to 547)	62.2 (−56 to 111)	41.0 (28.9 to 65.1)	43.8 (27.7 to 86.6)	9.4 (−21.5 to 5.6)
Nigeria, *n*=15	49.8	28-68	9	6	6	9	472.9 (143 to 1534)	571.2 (135 to 2178)	235.1 (−643 to 120)	543.7 (432 to 724)	523.4 (438 to 630)	77.9 (−119 to 159)	18.7 (8.5 to 43.1)	21.3 (7.3 to 57.4)	8.9 (−29.1 to 4.9)

BUN=blood urea nitrogen; D=diabetic; F=female; KB=Kibow Biotics; M=male; ND=nondiabetic; PL=placebo.

were lower in 15 patients (33%, no statistical significance). Please refer to Tables 3 and 4 for statistical analysis on these data and also details of respective standard deviation and relevant parameters.

Most subjects (85%, $P<0.05$; Tables 5 and 6) expressed a substantially higher perceived QOL during the KB treatment period (87% of ratings "good" [52%] or "very good" [35%], 11% "average," and 2% "poor"), in comparison with the placebo period (4% of ratings "very good," 50% "good," 37% "average," 7% "poor," and 2% "very poor"). Mild physical complaints, including bloating, flatulence, and diarrhea, were observed in 10 of the study patients. These symptoms were noticed only during the first 3 weeks of administration of probiotics and did not recur. Table 7 shows a summary of patients' improvements across all parameters measured.

Of 62 patients enrolled at all sites, 16 were lost to follow-up without suitable explanation. No patient withdrew because of objection to or adverse reaction after being fed bacterial microorganisms (probiotics). It is possible that some patients withdrew due to disappointment over not experiencing any immediate benefit from the trial product. In addition, most of the enrolled patients were already under different medications for their CKD conditions. As such, consuming additional trial products and not experiencing immediate drug-like effect might have prevented them from continuing their monthly visits during the study period. The number of patients who dropped out from each location were: USA (three), Argentina (five), Canada (three), and Nigeria (five). In all study sites, we also observed some increased levels of one or more biochemical markers with placebo and/or KB treatment periods. This could possibly be attributed to varied reasons such as dietary, fluid retention, or other unknown CKD symptoms or its disease progression status.

Table 3. Paired samples statistics based on average levels (across all sites).

		Mean	*n*	SD	SEM	Pair correlation	Significance
Pair 1	Creatinine KB	388.5217	46	229.84620	33.88897		
	Creatinine PL	414.0435	46	342.33615	50.47471	0.949	0.000
Pair 2	Uric acid KB	517.1304	46	99.43029	14.66020		
	Uric acid PL	504.5217	46	73.90557	10.89678	0.711	0.000
Pair 3	BUN KB	23.8217	46	12.01205	1.77108		
	BUN PL	25.8870	46	15.14002	2.23227	0.908	0.000

BUN=blood urea nitrogen; KB=Kibow Biotics; PL=placebo; SEM=standard error mean.

Table 4. Paired samples test of difference (across all sites).

					95% CI				Significance
	Paired differences	Mean	SD	SEM	Lower	Upper	t	df*	(2-tailed)
Pair 1	Creatinine KB – Creatinine PL	–25.52174	143.54600	21.16470	–68.14964	17.10616	–1.206	45	0.234
Pair 2	Uric acid KB – Uric acid PL	12.60870	69.99634	10.32040	–8.17765	33.39504	1.222	45	0.228
Pair 3	BUN KB – BUN PL	–2.06522	6.58723	0.97123	–4.02138	0.10905	–2.126	45	0.039

*Degrees of freedom.

BUN=blood urea nitrogen; KB=Kibow Biotics; PL=placebo; SEM=standard error mean.

Table 5. Quality of life ratings (by site and by treatment period).

Pt. no.	Canada			Argentina			Nigeria			USA		
	KB	PL	KB-PL	KB	PL	KB-PL	KB	PL	KB-PL	KB	PL	KB-PL
1	5	5	0	10	8	2	8	6	2	9	7	2
2	8	7	1	9	8	1	7	3	4	8	8	0
3	8	6	2	9	6	3	4	6	–2	8	5	3
4	7	6	1	10	7	3	8	6	2	6	6	0
5	6	5	1	7	7	0	9	7	2	9	6	3
6	7	6	1	8	7	1	9	6	3	9	7	2
7	7	7	0	9	8	1	8	7	1	8	7	1
8	7.5	7	0.5	10	7	3	7	5	2	8	7	2
9	10	5	5				8	7	1	10	8	2
10	10	7.5	2.5				8	7	1	8	7	1
11	10	9	1				9	9	0			
12	5	2	3				7	6	1			
13	8	7	1				7	5	2			
14							5	4	1			
15							7	4	3			
Average changes:			1.46			1.75			1.53			1.60

KB=Kibow Biotics; PL=placebo.

Table 6. Quality of life paired samples test.

	Paired differences	Mean	SD	SEM	95% CI		t	df	Significance (2-tailed)
					Lower	Upper			
Pair 1	Canada KB – Canada PL	1.46154	1.37631	0.38172	0.62984	2.29323	3.829	12	0.002
Pair 2	Argentina KB – Argentina PL	1.75000	1.16496	0.41188	0.77607	2.72393	4.249	7	0.004
Pair 3	Nigeria KB – Nigeria PL	1.53333	1.40746	0.36341	0.75391	2.31276	4.219	14	0.001
Pair 4	USA KB – USA PL	1.60000	1.08012	0.34157	0.72733	2.27267	4.392	9	0.002
Pair 5	All sites KB – All sites PL	1.54348	1.25533	0.18509	1.17069	1.91626	8.339	45	0.000

df=degrees of freedom; KB=Kibow Biotics; PL=placebo; SEM=standard error mean.

Table 7. Summary: percentages of patients showing improvement.

Site	No. patients	No. patients with decreased levels (%)			No. patients with improved quality of life ratings (%)
		Creatinine	Uric acid	BUN	
Argentina	8	4 (50)	4 (50)	4 (50)	7 (88)
Canada	13	7 (54)	4 (31)	13 (77)	11 (85)
Nigeria	15	5 (33)	5 (33)	7 (47)	13 (87)
USA	10	4 (40)	2 (20)	5 (50)	8 (80)
Totals	46	20 (43)	15 (33)	29 (63)	39 (85)

BUN=blood urea nitrogen

DISCUSSION

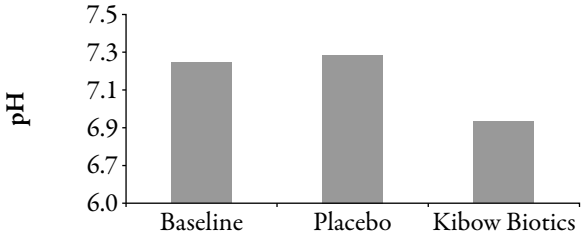
A food-grade, gram-positive bacteria in a probiotic formulation was previously found to be beneficial to rodents,[44,45] miniature pigs,[46] as well as cats[47] and dogs (Dr. Gary Van Engelenberg, DVM, CVA, Iowa Veterinary Acupuncture Clinic, Des Moines, IA, personal communications, January-June 2007) with renal failure.

While multiple probiotic products are now marketed, it is encouraging to appreciate that patients with substantive CKD are willing to participate in a study protocol that represented an initial human trial with an unknown outcome.

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 observed for 90 days may reflect intragut conversion of urea to ammonia, a source of nitrogen for multiple metabolic purposes, including additional microbial growth. One possible benefit of administering probiotic bacteria to azotemic subjects would be a reduction in the "ammonia burden," as bacterial metabolism substitutes for missing renal excretion of ammonia in renal failure. Fecal pH of the probiotic bacteria cohort (pH 6.94) was lower than the placebo cohort (pH 7.29) with a confidence interval of >95% (Figure 2). An alternative explanation for the lower stool pH observed in the cohort receiving probiotic bacteria might be that *Lactobacillus* in the mixture administered is a species known to generate an acidic environment due to production or generation of lactic acid.

A simple customized QOL questionnaire employing a subjective scale of 1-10 was also evaluated at all study sites. Subjects in all the study sites of this clinical trial were asked to maintain a diary throughout the study in which any unusual observations, including change in bowel movement frequency, gas, bloating, or any other discomforts, were recorded. In this context, each subject's self-assessment of QOL afforded insight into how oral ingestion of living bacteria might alter day-to-day behavior.

Figure 2. Observed fecal pH values.



The strength of this study lies in its documentation that a small group of subjects with CKD (*n*=46) safely completed a 6-month trial of probiotic bacteria without adverse incident, and with some indication of benefit in terms of moderating symptoms of kidney failure. Some subjects reported an improved QOL, which is a strong stimulus to further evaluate the concept that the bowel may partially substitute for missing kidney function when "activated" with probiotic bacteria.

LIMITATIONS

The small sample size imposed the main limitation in analysis of results in this pilot study. It also needs to be considered that reductions in plasma levels of creatinine, uric acid, and urea may sometimes result from a loss of appetite during bacteriotherapy, which may also be a possible limiting factor. However, lack of appetite was not reported by any study patients. It should also be noted that full nutritional assessment of study subjects was not affected, as this was a pilot-scale study undertaken with limited resources. In addition, the study design had patients serving as their own control, which

may be viewed as a study limitation. Therefore, both a larger patient cohort and an increased dose of administered probiotic formulation will be a major objective of future planned derivative studies. Lastly, in future trials, we intend to use a SF36 questionnaire, which will be a more appropriate tool for assessing QOL as opposed to the custom-made questionnaire with a subjective scale of 1-10 that was used for this study.

CONCLUSION

The main outcomes of this preliminary trial include a significant reduction of BUN, enhanced well-being, and absence of serious adverse effects, all of which support the use of the chosen probiotic formulation for bowel-based toxic solute extraction. QOL and BUN levels showed significant difference in outcome ($P<0.05$) between placebo and probiotic treatment periods at all four sites (46 patients). Further evaluation of this probiotic formulation will include a dose-escalation trial in a similar prospective, placebo-controlled, and double-blind study.

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