Effects of Kibow Probiotic Supplementation Renadyl™ on Uremic Toxins in Hemodialysis Patients

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Abstract

Our prior studies in patients with CKD 3-4 (n=31) given Renadyl™, a safe proprietary probiotic dietary supplement that metabolizes nitrogenous wastes in the bowel, at a dose of 90-270 B CFU per day, over a 4 month period showed that BUN, creatinine and K+ levels declined. We now conducted a prospective double blind cross over trial with placebo and Renadyl™ in 26 stable CKD patients on hemodialysis. Dosage administered was 180 B CFU per day, given in 3 divided doses. Our primary aim was a 20% reduction in BUN levels over an 8 week period. Patients' dialysis prescriptions were unchanged. Our secondary aim was to see if there would be changes in WBC count, C-reactive protein (CRP), total and/or free serum concentrations of indoxyl sulfate, indole acetic acid, p-cresyl sulfate, hippuric acid, serum pentosidine, 3-carboxyl-4-methyl-5-propyl-2-furan-propanoic acid (CMPF), uric acid and beta-2 microglobulin. Solutes were measured by HPLC and ELISA. QoL changes were assessed by a modified SF-36 questionnaire. Patient adherence was assessed by pill count and stool culture to verify probiotic growth during study and absence during placebo period. Data were analyzed using ANOVA for a crossover study with a mixed model methodology in SAS to account for treatment, period and sequence effects. Administration of probiotics was safe and showed a decline in WBC counts ($6.02x10^3$/uL to $5.51x10^3$/uL, p=0.05) and total indoxyl glucuronide (0.76mg/dL to 0.65mg/dL, p= 0.05) and a trend towards reduction in CRP (13.72mg/dL to 5.11mg/dL, p= 0.07). Other chemicals and QoL were unchanged. Administration of Kibow Probiotic Renadyl™ in ESRD patients is safe and showed a protective effect by the trend to reduce markers of inflammation. Further investigation in a larger population or at a higher dose might yield mechanistic insights into the probiotic effects on the inflammatory cascade of uremia.

Objectives

Previous studies had shown that Renadyl™ was able to improve quality of life and decrease BUN in patients with ESRD. However, the mechanism of action and what toxins are also reduced are unknown. Our goal is to identify uremic toxins or markers of inflammation that are decreased in response to the Probiotic Renadyl™.

Methods

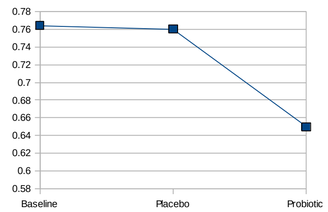
Patients were assigned to take either the placebo or Renadyl™ first for 8 weeks, followed by a washout period of 8 weeks and finishing with 8 weeks of the placebo or Renadyl™ (depending on which was taken first). Each patient's blood samples were taken at the first visit, after finishing 8 weeks of placebo, and after finishing 8 weeks of Renadyl™.

The sera were used to measure C-reactive protein (CRP), total and/or free serum concentrations of indoxyl sulfate, indole acetic acid, p-cresyl sulfate, hippuric acid, serum pentosidine, 3-carboxyl-4-m. To determine the total serum concentration, 75 µL of sample was diluted with 195 µL of HPLC water followed by heating at 95° C for 30 min. After heating, the samples were placed on ice for 10 minutes. Subsequently, samples were filtered through a molecular filter with a molecular weight cut-off of 30000 Da using Amicon Ultra 0.5 mL Filters. To measure the free fraction, untreated serum samples were filtered through the Amicon Ultra Filters prior to heating. In order to correct for system performance variations, 25 µL of fluorescein (50 mg/L) was added to 225 µL of ultrafiltrate as internal standard. Subsequently, this was transferred to an autosampler vial and 50 µL thereof was injected on the column.

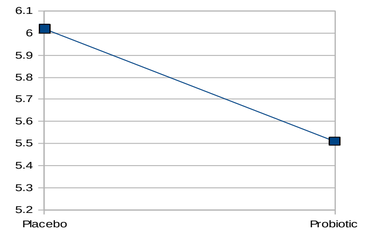
The instrumentation for the HPLC analyses consisted of a Waters Alliance 2695 device (Waters, Zellik, Belgium) and two detectors in series; a Waters 996 photodiode array detector (PDA) and a Waters 2475 fluorescence detector (FLD). The separation was performed at room temperature on a reversed-phase XBridge C8 column (3.5 µm, 150 mm x 4,6 mm, Waters) with an Ultrasphere ODS guard column (5 µm, 5 mm x 4.6 mm, Beckman Instruments). The mobile phase consisted of a 50 mM ammonium formate buffer (mobile phase A, pH 3.0) and methanol (mobile phase B). A gradient elution at a flow of 1 mL/min was performed with an initial composition of 100% phase A and held at this composition for 3 min. Then, this increased to 100% B in 31 min and this composition was held for 3 min, and finally a re-equilibration. For uric acid, hippuric acid and CMPF chromatograms were extracted from the PDA data at 300 nm, 245 nm and 254 nm, respectively. Fluorescence excitation and emission wavelengths were optimized for the other compounds: λ_{ex} = 272 nm and λ_{em} = 374 nm for indoxylsulfate and indoxylglucuronide, λ_{ex} = 264 nm and λ_{em} = 290 nm for p-cresylsulfate and p-cresylglucuronide, λ_{ex} = 272 nm and λ_{em} = 340 nm indole acetic acid, and λ_{ex} = 443 nm and λ_{em} = 512 nm for the internal standard. Five point calibration curves were generated. Good linearity was observed for all compounds. For the regression calculation a weighing factor of 1/x was used for all data points. Data analysis was done using SAS.

Results

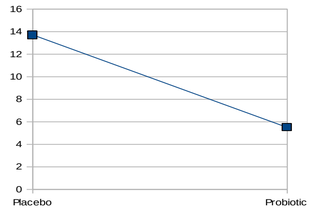
Indoxyl glucuronide Levels



WBC Count



CRP Levels



These graphs show the decline in WBC count, CRP levels, and indoxyl glucuronide levels in patients taking Probiotics when compared to placebo. Other measured chemicals show no significant change.

Conclusions

Our data show that Renadyl™ reduces the levels of CRP, the levels of total indoxyl glucuronide, and WBC counts. This suggests that Renadyl™ exerts some protective effect by reducing the levels of inflammation in the form of WBC counts and CRP. Future investigations using a larger population may yield mechanistic insights on Probiotic's effects on uremia and the inflammatory cascade.

References

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