Effect of Kibow Probiotic Renadyl™ on NF-κB Levels in Hemodialysis Patients

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Abstract

End-stage renal disease (ESRD) is when the kidneys are unable to function effectively and requires dialysis. As a consequence of this condition, uremic toxins build up and this has been associated with increased inflammation. NF-κB is an inflammatory marker that has been shown to increase in uremia. Previous studies showed that Renadyl™, a safe proprietary dietary supplement, decreased BUN, creatinine, and K+. This study was done to see if Renadyl™ has an effect on NF-κB in ESRD patients on hemodialysis. We conducted a prospective, double-blind crossover trial with placebo and RenadylTM in 26 ESRD patients on hemodialysis. Each patient had 3 time points measured: Baseline, after taking probiotics for 8 weeks, and after taking placebo for 8 weeks. Perusal blood mononuclear cells (PBMC) were extracted from the patient blood samples using ficoll hypaque and NF-κB levels were assessed using the TransAM p65 ELISA kit (Active Motif). Viability of cells was assessed using trypan blue exclusion. Patient adherence was assessed by pill count and stool culture to verify Probiotic adherence. Flow cytometry was used to evaluate the number of activated lymphocytes. The lymphocytes were isolated from whole blood using ficoll hypaque and NF-κB levels were assessed using the protocol from the kit. The wells contained DNA with the consensus sequence specific for activated NF-κB. 200L of the samples (corresponding to extracts from 250,000 cells) and 30L of binding buffer were added to each well on the assay plate. This was allowed to incubate at room temperature for 1 hour with light agitation. The wells were washed with 200L 1x wash buffer 3 times. The primary antibody solution contained the antibody to the NF-κB DNA complex and was prepared by diluting the antibody solution 100-fold in the kit’s antibody dilution buffer. 100L of this solution was added to each well and the samples were incubated at room temperature for 1 hour without agitation. The solutions were removed and the wells were washed 3 times with 200L 1x wash buffer. The secondary antibody was specific for the primary antibody-NF-κB complex and was conjugated with horseradish peroxidase. This solution was prepared by diluting the antibody solution 1000 fold in the kit’s antibody dilution buffer. 100L of this solution was added to each well and the samples were incubated at room temperature for 1 hour without agitation. The solutions were removed and the wells were washed 4 times with 200L 1x wash buffer. 100L of substrate solution was added to each well and the wells were allowed to incubate at room temperature until the wells generated a medium-blue color. Then 100L of stop solution was added to each well and the plate was read at 450nm. The data were analyzed using SAS.

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 serum was extracted from the blood and this was used to determine the NF-κB levels.

The TransAM p65 NF-κB assay kit purchased from Active Motif was used to perform the assays for the samples. The lymphocytes were isolated from whole blood using ficoll hypaque to form the density gradient and centrifuged. An aliquot of the cells extracted was used for lysis. The nuclear content from the aliquot was extracted using the protocol from the kit. The final solution was diluted to 12500 cells/ul. Using the cell lysis buffer combined with the protease inhibitor cocktail. The cell extracts were stored at -80°C. The binding buffer, wash buffer, and antibody dilution buffers were prepared using the protocol from the kit. The wells contained DNA with the consensus sequence specific for activated NF-κB. 200L of the samples (corresponding to extracts from 250,000 cells) and 30L of binding buffer were added to each well on the assay plate. This was allowed to incubate at room temperature for 1 hour with light agitation. The solutions were removed and the wells were washed 3 times with 200L 1x wash buffer. The secondary antibody was specific for the primary antibody-NF-κB complex and was conjugated with horseradish peroxidase. This solution was prepared by diluting the antibody solution 100-fold in the kit’s antibody dilution buffer. 100L of this solution was added to each well and the samples were incubated at room temperature for 1 hour without agitation. The solutions were removed and the wells were washed 3 times with 200L 1x wash buffer. The secondary antibody was specific for the primary antibody-NF-κB complex and was conjugated with horseradish peroxidase. This solution was prepared by diluting the antibody solution 1000 fold in the kit’s antibody dilution buffer. 100L of this solution was added to each well and the samples were incubated at room temperature for 1 hour without agitation. The solutions were removed and the wells were washed 4 times with 200L 1x wash buffer. 100L of substrate solution was added to each well and the wells were allowed to incubate at room temperature until the wells generated a medium-blue color. Then 100L of stop solution was added to each well and the plate was read at 450nm. The data were analyzed using SAS.

Conclusions

Our results show that the NF-κB pathway is not modulated by the effects of the probiotic, Renadyl™. This also shows that Renadyl™ is not harmful as it does not induce active inflammation, although studies on other markers may be needed to determine the probiotic’s mechanism of action.

References