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

Subodh J. Saggi[1], Eli A. Friedman[1], Lorraine L.a Thomas[1], Natarajan Ranganathan[2], Pari Ranganathan[2], Gary R. Briefel[1], Mary C. Mallappallil[1], Usha N. Vyas[2], Bohdan Pechenyak[2], Peter Liang[1], David Hochman[1] and Allen J. Norin[1].

[1]SUNY Downstate Medical Center, Brooklyn, NY and [2]Kibow Biotech, Newton Square, PA

Abstract

End-stage renal disease (ESRD) is when the kidneys are unable to function effectively to sustain life 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. Peripheral blood mononuclear cells (PBMC) were extracted from the patient blood samples using ficoll hypaque and NF-κB levels were assayed 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 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 effect. NF-κB levels were 0.407U/uL when treatment with Probiotic was first in sequence and 0.409U/uL when placebo was administered first. There were no differences in least square means between placebo and Probiotic (p=0.9407).These results indicate that NF-κB pathway is not activated and not modulated under the effect of Probiotic Renadyl™. This further suggest that the administration of probiotics to ESRD patients in itself is not harmful as the neither this pathway is activated nor suppressed as activation of this pathway is required in the settings of active infections.

Objectives

The Probiotic, Renadyl™, had been shown to reduce uremic toxins. One of the markers of inflammation that is increased in the presence of uremic toxins is NF-κB. The goal of this study is to determine if Renadyl™ exerts anti-inflammatory effects by decreasing NF-κB. We hypothesized that the levels of NF-κB would decrease with administration of Renadyl™.

Methods

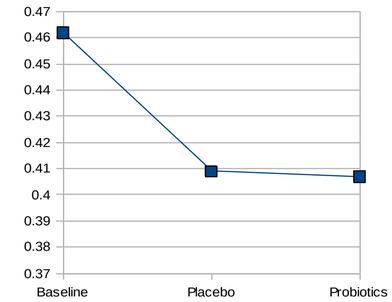
Patients were assigned to take either the placebo or Renadyl™ first for 8 weeks, followed by a washout period of 8 weeks and finishing with 8 weeks of the placebo or Renadyl™ (depending on which was taken first). Each patient's blood samples were taken at the first visit, after finishing 8 weeks of placebo, and after finishing 8 weeks of Renadyl™. The 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. 20uL of the samples (corresponding to extracts from 250,000 cells) and 30uL of binding buffer were added to each well on the assay plate and this was allowed to incubate at room temperature for 1 hour with light agitation. The wells were washed with 200uL 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 1000-fold in the kit's antibody dilution buffer. 100uL 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 200uL 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. 100uL of this solution was added to each well and the samples were allowed to incubate for 1 hour at room temperature without agitation. The solutions were removed and the wells were washed 4 times with 200uL 1x wash buffer.

100uL 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 100uL of stop solution was added to each well and the plate was read at 450nm. The data were analyzed using SAS.

Results



Mean baseline NF-κB levels were 0.462 U/ul. Mean levels were 0.409U/ul after taking Probiotics and 0.407U/ul after taking placebo. The results are not significant (p=.9407).

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

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