Probiotic supplements prevented oxonic acid-induced hyperuricemia and renal damage

Fernando E. García-Arroyo[1], Guillermo Gonzaga[1], Itzel Muñoz-Jiménez[1], Mónica G Blas-Marron[1], Octaviano Silverio[1], Edilia Tapia[1], Virgilia Soto[2], Natarajan Ranganathan[3], Pari Ranganathan[3], Usha Vyas[3], Anthony Irvin[3], Diana Ir[4], Charles E. Robertson[4], Daniel N. Frank[4], Richard J. Johnson[5] and L. Gabriela Sánchez-Lozada[1*]

[1] Laboratory of Renal Physiopathology, INC Ignacio Chavez, Mexico City, Mexico. [2] Dept. of Pathology, INC Ignacio Chavez, Mexico City, Mexico.[3] Kibow Biotech, Inc., Newtown Square, PA, USA.

[4] Division of Infectious Diseases, University of Colorado, Aurora CO, USA [5] Renal Diseases and Hypertension, University of Colorado, Aurora CO, USA.

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Introduction:

Hyperuricemia is often present in the population, with some studies reporting a prevalence as high as 21% in men and women. While the presence of hyperuricemia in the absence of gout has often been described as"asymptomatic", recent studies suggest hyperuricemia may have a contributory role in metabolic and cardiovascular diseases. Extrarenal excretion of uric acid via the intestine accounts for up to one-third of its total excretion . Uric acid is secreted into the gut where it is rapidly metabolized by bacterial microbiota . Moreover, it was recently shown that gouty patients have a significantly different intestinal microbiota in comparison to normouricemic subjects , a finding that suggests an interaction between the microbiota and intestinal UA metabolism and excretion that could potentially modulate serum uric acid levels.
Current therapy for lowering serum UA includes inhibitors of xanthine oxidase (allopurinol, febuxostat), recombinant uricase (rasburicase) and uricosuric agents (probenecid). Nevertheless, all these drugs may produce undesired secondary effects. Therefore, the development of alternative therapeutic strategies to reduce UA concentrations would be useful. Hence, a natural inexpensive, safe product as a non-drug pro/prebiotic dietary supplement could be an attractive alternative biotherapeutic product for hyperuricemia/gout applications.

Aim:

This pilot study was designed to evaluate the potential of two probiotic supplements to lower systemic uric acid concentrations. Secondary objectives were to evaluate whether hypouricemia was accompanied by a therapeutic benefit on the hyperuricemia-induced renal damage and hypertension. Finally, we profiled fecal microbiota in order to assess the effects of hyperuricemia and probiotic supplementation on bacterial community structure

Learning Objectives:

1. To discuss the probabale health advantages of using probiotics to reduce serum uric acid levels instead of drugs.
2. To discuss the positive impact of treating hyperuricemia for preventing renal and cardiovascular damage.
3. To discuss the suitability of performing clinical studies.

Methods:

Thirty male Wistar rats were ordered from Envigo Mexico (Mexico City, Mexico), and given 5 days to acclimate to the housing facility prior and be trained for baseline systolic blood pressure (SBP) measurement. Rats were housed in micro barrier system cages and given access to food and water ad libitum during acclimation. .Animals were monitored on a daily basis for health status. Baseline urine and fecal samples were collected by placing rats in metabolic cages (Tecniplast. Varese, Italy) for 16 h with food and water ad libitum. SBP was measured by tail-cuff manometry in conscious animals previously accustomed to this procedure (NIBP System IN125/R. ADInstruments Inc. Dunedin, New Zealand). Three consecutive measurements were recorded, and the mean reported. After SBP quantification a sample of blood was taken from the tail vein (600 µL), and plasma stored at -20˚C until further processing.

Five groups of 6 rats each were studied. Oxonic acid, potassium salt was dosed daily by gavage using flexible polyethylene tube and a syringe in morning hours for a total of 5 weeks, including weekends.

Diets: Rat Chow AIN-93 (Purified Diets for laboratory rodents) was purchased from Dyets Inc. Bethlehem PA. Two probiotic containing formulas were prepared and stored at -20C .The compositions of the formulas were : Placebo–Cream of wheat. Formula 1- L acidophilus KB27 (5.0 B CFU/day), L rhamnosus KB79(5.0 B CFU/day), Xylooligosaccharide-50.0 mgs per day .Formula 2- L acidophilus KB27 (5.0 B CFU/day), L rhamnosus KB79(5.0 B CFU/day), Xylooligosaccharide-50.0 mgs per day, curcumin-25.0 mgs/day .The formulas were mixed into the rat chow and made into 5.0-gram balls. The following groups were included: C = Control group. Normal healthy rats receiving normal regular diet HU-ND = Oxonic acid-induced hyperuricemia receiving normal regular diet HU-P = Oxonic acid-induced hyperuricemia receiving placebo containing diet HU-F1 = Oxonic acid-induced hyperuricemia receiving probiotics formula 1 containing diet. HU-F2 = Oxonic acid-induced hyperuricemia receiving probiotics formula 2 containing diet.

Oxonic acid dosing and probiotics feeding were started at the same time point. No adverse events were observed during the 5-week follow-up, and all rats reached the end of the study.

Systolic blood pressure measurements, urine collection, and blood samples were obtained at 3 and 5 weeks, at this latter point fecal samples were also collected. At the end of the study, rats were sacrificed by deep anesthesia with inhaled isoflurane and exsanguination via abdominal aorta with a heparinized syringe. The collected blood was centrifuged, and plasma separated and frozen until further analysis. Immediately, the kidneys were washed by perfusion with cold PBS and right kidney excised separated in cortex and medulla and stored in liquid nitrogen until further processing. The left kidney was fixed by perfusion for histological analysis. Samples of the small intestine (ileum) were also taken and stored in liquid nitrogen.

Uric acid in plasma and urine were measured using a commercial enzymatic kit (Sekisui, Diagnostics. Charlottetown, PE. Canada). 5-week plasma samples were in addition, tested using a colorimetric assay (Quantichrom Uric Acid Assay, BioAssay Systems. San Francisco CA, USA).
Urine TBARS and products of nitric oxide metabolism in plasma and urine (nitrates and nitrites) were measured with commercial kits (Cayman Chemical, Ann Arbor, Michigan. USA) Plasma and urine creatinine were measured with a commercial kit (SpinReact. Girona, Spain) and creatinine clearance calculated.

Urine NAG activity was determined using 4-nitrophenyl-N-acetyl-beta-D-glucosaminide as substrate.

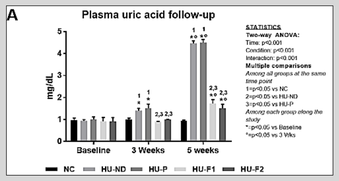
Renal cortex oxidative stress: The determination of carbonyl groups in the proteins of renal cortex homogenates was measured using the reaction with 2,4-Dinitrophenylhydrazine (DNPH) . For the 4-HNE assay, 50 mg of kidney cortex were homogenized in ice-cold phosphate buffered saline (PBS), and measured by colorimetric assay. The results were expressed as nmol of 4-HNE/mg protein.

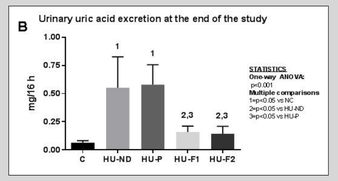
Renal cortex uric acid content: Uric acid was extracted from the renal cortex. Uric acid concentration was measured with a commercial enzymatic kit (Sekisui, Diagnostics. Charlottetown, PE. Canada) and corrected by protein concentration (Bradford method).

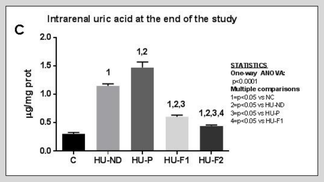
Microbiome analysis DNA was extracted from fecal samples using the QIAamp PowerFecal Kit (QIAGEN, Carlsbad, CA). Bacterial profiles were determined by broad-range amplification and sequence analysis of 16S rRNA genes. Illumina paired-end sequencing was performed on the Miseq platform using a 600 cycle version 3 reagent kit.

Statistical analysis Values were expressed as the mean ± standard deviation (SD). Differences between groups were evaluated by two-way ANOVA or one-way-ANOVA with Tukey's correction for multiple comparisons. The R and Explicet software packages were used for all microbiome analyses and figure generation. Differences in overall community composition between treatment groups were assessed using the permutation-based multiple analysis of variance (PERMANOVA) test of Bray-Curtis dissimilarities, as implemented in the R vegan package. P-values were obtained through 1,000,000 permutations. Individual OTUs that differed in relative abundance or prevalence between groups were identified using a non-parametric KruskalWallis test. Measures of alpha-diversity (Chao1, evenness, Shannon complexity) were estimated using Explicet (v2.10.5,) through 1,000 replicate re-samplings at the rarefaction point of 54,698 sequences; between-group differences in these indices were assessed by ANOVA.

Results:Primary Endpoints







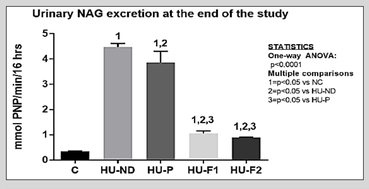
1) Plasma uric acid levels were reduced in the probiotic fed groups at week 5. Although the plasma uric acid in the probiotic fed groups were higher at the end of the study as compared to baseline values,the levels were significantly lower compared to the oxonic acid controls and the placebo fed group.(A). Both formulas prevented the rise in uric acid excretion resulting from oxonic acid induced hyperuricemia significantly(B).Intrarenal uric acid accumulation was also reduced partially but significantly in the probiotic fed groups.

Results: Secondary Endpoints

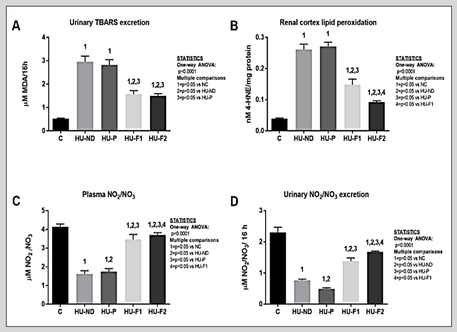
	Control	HU+ND	HU+P	HU+F1	HU+F2
Baseline	124±11	128±5	119±11	128±4	123±10
3 weeks	119±9	136±10[1]	133±3	124±9	125±5
5 weeks	126±7	135±7	138±4*	118±8[2,3]	121±6[3]

Statistics:
Two-way ANOVA Time = ns; Condition p<0.001; Interaction p<0.01
Multiple comparisons
Among all groups at the same time point: 1 = p<0.05 vs C; 2 = p<0.05 vs HU-ND; 3 = p<0.05 vs HU+P.
Among each group along the study
* = p<0.05 vs Baseline

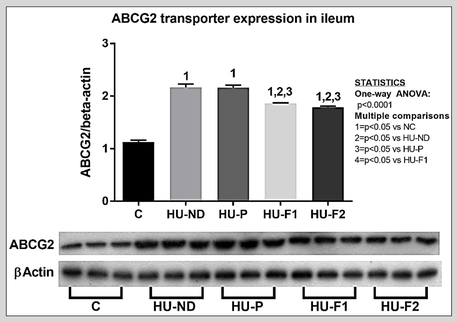
2) Systolic blood pressure rose in the oxonic and placebo fed groups. In the probiotic fed groups with and without Curcumin the increase in the SBP was prevented from rising at the final SBP were lower though not significant at the end of the study.



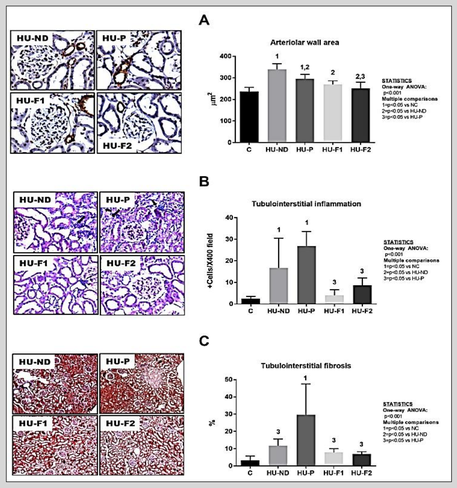
3) Urinary N-acetyl-beta-D-glucosaminidase (NAG) urinary activity which is a marker of tubular injury was also lower in the probiotic groups. Feeding with the probiotic formula partially prevented the rise of NAG activity at the end of the study.



4) Thiobarbituric acid reactive species (TBARS) urinary excretion is a measure of systemic lipid peroxidation secondary to oxidative stress. Hyperuricemic groups receiving normal diet or placebo had a 5-time increment in the excretion of urinary TBARS. The feeding with probiotic formulas partially but significantly ameliorated that effect.
Lipid peroxidation was significantly increased in the renal cortex. This oxidative stress was partially prevented by in the probiotic fed groups with the second formula containing curcumin showing a significantly better effect.
Hyperuricemia induced a marked reduction in plasma and urinary NO2/NO3 at the end of the study. Probiotic feeding partially rescued nitric oxide byproducts
plasma and urinary levels, and this effect was better observed in the rats fed the formula containing curcumin.



5) In humans, some polymorphic variants of the transporter BCRP (also known as ATP-binding cassette transporter, sub-family G, member 2, ABCG2), which secretes UA into the intestine, result in decreased transporter activity, elevated serum uric acid (SUA) and increased incidence of gout.
Probiotic supplementation partially reduced the overexpression of the ABCG2 transporter.



6) **Histopathological findings :**
Renal histologic analysis performed at the end of the study (after 5 weeks of oxonic acid exposure) documented an afferent arteriolopathy in oxonic acid-treated rats with hyperuricemia (defined as an increase in arteriolar wall area, Fig A), as well as tubulointerstitial inflammation (Fig B) and fibrosis (Fig C). These three alterations reached statistical significance in the group that received placebo, while in the normal diet hyperuricemic group, the fibrosis did not reach statistical significance. Probiotic formula feeding prevented those alterations, and the effect was greater observed when compared versus the placebo group.

Conclusions/Summary

We demonstrate for the first time the ability of probiotics containing uricolytic bacteria to lower serum uric acid in hyperuricemic animals with beneficial consequences on blood pressure and renal disease. We suggest clinical studies are needed to evaluate this approach for treating hyperuricemia in humans.

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