The effect of prebiotics on intestinal flora and renal function: attempts to inhibit AKI

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Abstract

The number of patients with chronic kidney disease (CKD) is increasing. Furthermore, the number of patients with CKD progression from acute kidney injury (AKI) is increasing. Thus, the establishment of a reliable treatment for kidney injury is an urgent issue. In this study, we investigated the effects of lactulose, prebiotics, on the intestinal flora and renal function in rat model of renal injury.

Renal injury was induced by 5/6 nephrectomized rats and fed with a diet supplemented with lactulose for 8 weeks. The renal injury rats showed markedly increased albuminuria and decreased creatinine clearance compared to control rats. In contrast, these renal dysfunctions improved in the lactulose-treated group (Albuminuria, control; 73.3±31.3, Renal injury; 577.0±274.5, Renal injury + lacturose; 426.0±169.4 μg/mg creatinine. Creatinine clearance, control; 5.06±2.74, Renal injury; 2.24±0.73, Renal injury + lacturose; 3.24±1.14 mL/min/kg BW). In addition, renal injury rats showed glomerular hypertrophy, which was reduced in the lactulose-treated group. The results of analysis of bacterial DNA extracted from feces using a next-generation sequencer showed an increase Proteobacteria and Lentisphaerae of intestinal flora in the renal injury rats. On the other hand, the percentage of Bifidobacteria in the lactulose-treated group was markedly increased.

These results suggest that there may be a protective mechanism for renal function through the increase of Bifidobacterium bifidum and improvement of intestinal flora by lactulose administration.

Methods and Materials

Animal studies

The SD rats were randomly divided into a sham group (n = 9) and a two-step standard 5/6 NX surgery group (n = 39) after a week of environmental acclimation. In the 5/6 NX group, two-thirds of the right kidney (upper and lower poles) was resected from SD rats under isoflurane anaesthesia. Recovery was maintained for 1 week, after which total NX of the left kidney was performed. One week following the second surgery (the uremic rats model established), 5/6 NX rats were randomly divided into with (n=21) or without lacturose (n=18) for 8 weeks.

Measurement of urinary albumin

Albuminuria was measured by Nephrat (Exocell Inc., Philadelphia, PA, USA) using 24-h urine collection samples from animals housed in individual metabolic cages.

Histological studies

Kidney sections for light microscopy analysis were fixed in 4% paraformaldehyde phosphate buffer. Sections were stained with periodic acid-Schiff. Glomeruli were digitally photographed and the images were imported to ImageJ software (National Institutes of Health, Bethesda, MD, USA; https://imagej.nih.gov/ij/) and analysed morphometrically.

DNA fragmentation analysis

DNA fragmentation was measured by quantitation of cytosolic oligonucleosome-bound DNA using an ELISA, according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN, USA).

Real-time PCR analysis

Total RNA was isolated from the glomeruli using an RNAeasy microcolumn with DNase treatment (Qiagen, Valencia, CA, USA). Quantification of RNA was performed with the NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA). mRNA expression in the glomeruli was evaluated by a SYBR green procedure (Applied Biosystems, Foster City, CA, USA). Amplification and detection were performed using the Step One Plus system (Applied Biosystems). Expression levels were normalized to levels of GAPDH.

Results

Table1: Physiological characteristics of the experimental groups

	sham	Nx	Nx + lactulose
albuminuria (μg/mg Cr)	73.3±31.3	577.0±274.5**	426.0±169.4$^{**\,\#}$
urine creatinine (mg/dL)	188.9±40.9	48.9±14.5**	71.1±18.7$^{**\,\#\#}$
plasma creatinine (mg/dL)	0.50±0.20	0.95±0.38**	0.62±0.21$^{\#\#}$
creatinine clearance (mL/min/kg BW)	5.06±2.74	2.24±0.73**	3.24±1.14$^{*\,\#}$

Mean±SD $^{*}p<0.05$, $^{**}p<0.01$ vs. sham, $^{\#}p<0.05$, $^{\#\#}p<0.01$ vs. 5/6 nephrectomy

Figure1: Effect of prebiotics on mesangial expansion

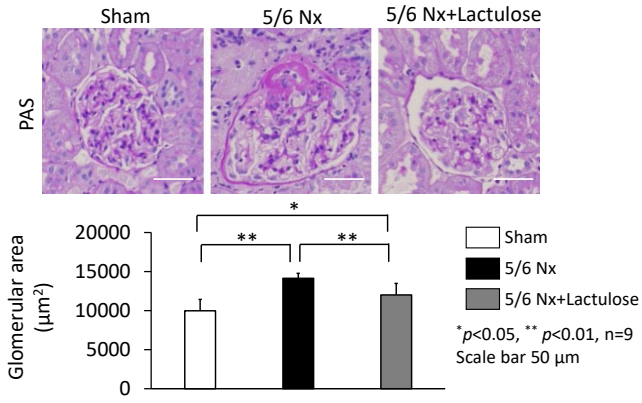
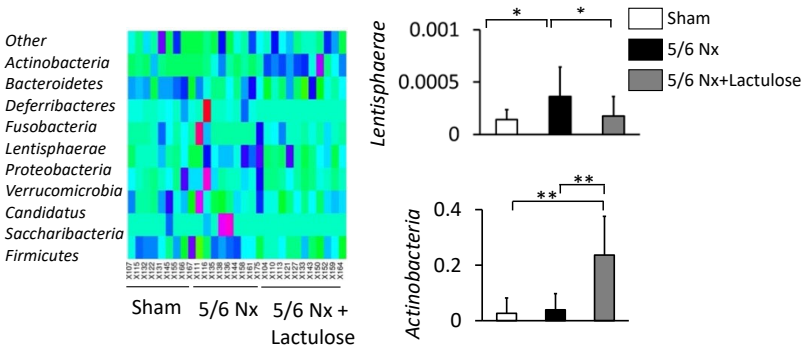


Figure2: Effect of prebiotics on microbiome



Conclusions & Discussion

Lactobacillus and Prevotella are thought to decrease in chronic kidney disease and renal failure. However, the evaluation of intestinal bacteria has not been consistent due to differences in assessment methods, race, age, and other factors. Therefore, inflammation, oxidative stress, enteroendocrine hormones, and short-chain fatty acids have been proposed to explain the relationship between intestinal flora and these pathological conditions.

Contrary to initial expectations, Lactobacillus and Prevotella tended to increase in rat with renal failure. As a possible cause, this study used a general phylogenetic analysis method based on 16S rRNA region amplification. However, since this method amplifies all 16S rRNA in the environment, there is a possibility that dead RNA remnants of Lactobacillus and Prevotella are also amplified.

In conclusion, our findings indicate that prebiotics had a clear beneficial effect on renal function and pathology by restoring the balance of the microbiome.

THE 28TH INTERNATIONAL CONFERENCE ON
ADVANCES IN CRITICAL CARE NEPHROLOGY
AKI & CRRT 2023
MARCH 29 - APRIL 1 SAN DIEGO, CALIFORNIA