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Intestinal Microbiota in Experimental Acute Kidney Injury

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Abstract
Recent studies have demonstrated an important role played by gut microbiota in maintaining intestinal homeostasis and host immune system function. Gut microbiota have been studied in experimental acute kidney injury (AKI) using different mice and rat models exposed to either ischemia or cisplatin-mediated tubular injury. Differences in inflammatory markers and severity of AKI have been observed between germ-free mice, wild-type mice, and mice treated with antibiotics or specific bacteria. Interventions modifying the gut microbiota after experimental AKI have had either beneficial or harmful effects on kidney tubular injury and recovery. These findings provide strong evidence for a modulatory role of gut microbiota during AKI. Ischemic and cisplatin-induced AKI have distinct stool microbial signatures based on 16s sequencing. Future in-depth studies exploring the mechanisms of how the microbiota influence AKI and development of feasible therapeutic options have the potential to improve outcomes in clinical AKI.

Introduction

Acute kidney injury (AKI) is a complex process with many key pathophysiologic processes including inflammation, cell death pathways, reactive oxygen damage, epigenetic changes, and various other mechanisms [1]. In addition, considerable advances have been made in demonstrating the important role of crosstalk between the kidney and distant organs during AKI [2]. While earlier studies demonstrated important communication between the kidney with the lung, heart, and brain, more recent data have revealed novel, unexpected relationships between the gut microbiome and kidney during AKI. This paper briefly summarizes the data on this topic.

Gut Microbiota and Pathogenesis of AKI

Trillions of bacterial microbes reside in the human gut and constitute the gut bacterial microbiota, predominantly compromised by phyla *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria*. A healthy gut microbiota composition plays a vital role in maintaining intestinal homeostasis and immune system function. An imbalance in the gut microbiota (gut dysbiosis) has been

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Table 1. Interventions influencing gut microbiota and kidney function in AKI

Intervention	Changes in metabolites and inflammatory markers	Changes in gut bacteria	Tubular injury	Author (year)
1) 6-week GF mice vs. control (Swiss Webster mice) at baseline and 24 h after IRI 2) Conventionalizing of GF mice after IRI	– Higher baseline NKT cells pre-IRI and higher CD8 T cells in GF mice post-IRI compared with control mice – Differences in NKT cells and cytokines abolished after conventionalizing	Not measured	– Worse in GF mice[1] – Improved after conventionalizing[2]	Jang et al. [3], (2009)
Acetate supplementation 30 min before ischemia and during reperfusion	– Low macrophages CD11b+, F4/80+ – Lower dendritic cells CD11c+, CD40+	Not measured	Improved[2]	Andrade-Oliveira et al. [4], (2015)
1) IRI after gut microbiota depletion with neomycin, metronidazole, ampicillin, and vancomycin 2) Transplanting antibiotic-treated mice with microbiota from untreated mouse	– Lower IL6, TNF-alpha, and chemokines MCP-1 and MCP-2 alpha in F4/80+ macrophages in antibiotic-depleted mice compared to control mice – Increase in F4/80+ macrophage expression after fecal transplantation	Not measured	– Less injury in antibiotic-treated mice[2] – More injury after fecal transplantation[1]	Emal et al. [6], (2017)
1) 16S rRNA sequencing of mouse feces on days 0, 2, and 10 after IRI vs. sham 2) Ampicillin, neomycin, metronidazole, vancomycin, and gentamicin 12 weeks before IRI in B6 mice 3) D-serine administration	– Inflammatory markers not measured – Enlargement of cecum in antibiotic-treated mice – Higher D-serine to L-serine ratio in feces, plasma, and kidney after IRI compared with sham surgery – D-serine low-dose administration of 20 mM decreases F4/80+ cells	Microbiota richer with higher *Lactobacillus, Clostridium*, and *Ruminococcus* and lower *Bifidobacterium* and *TM7* in IRI vs. sham group	– Higher tubular injury in antibiotic-treated mice[1] – Reduced tubular injury in D-serine-treated mice[2]	Nakade et al. [5], (2018)
1) GF mice colonized with post-IRI microbiota 2) 16S rRNA in Sham vs. IRI vs. b/l nephrectomy groups 3) Ampicillin, neomycin, metronidazole, and vancomycin 2 weeks before IRI	– Expansion of IL-17A+ CD4 cells, reduced fecal SCFA, higher blood endotoxin levels, TNF-alpha, and interferon gamma after colonization with post-IRI microbiota – Reduced TH1, TH17 response and increased regulatory T cells and M2 macrophages after antibiotics	– Increase in *Escherichia*, *Enterobacter*. Decrease in *Lactobacillus*, *Ruminococcaceae, Faecalibacterium*, *Lachnospiraceae* in IRI and nephrectomy vs. sham mice – Increase *Lactobacilli* and SCFA after antibiotics	– Higher tubular injury after colonization with post-IRI microbiota[1] – Reduced tubular injury after antibiotics[2]	Yang et al. [7], (2020)
Metabolites (mass spectroscopy) and fecal microbiome (16S RNA) 48 h after IRI	Increase in 31 acetylcarnitines and decrease in 3 amino acids (tyrosine, tryptophan, and proline) after IRI	*Rothia* and *Staphylococcus* species positively correlated and *Prevotella copri, Faecalibacterium prausnitzii*, and *Coprococcus eutactus* inversely correlated with rise in creatinine		Andrianova et al. [8], (2020)
Ampicillin, vancomycin, and levofloxacin for 14 days before IRI	Lower glucose and pyruvate levels in antibiotic-treated mice (vs. control) after IRI	Reduced SCFA and 16S rRNA quantity in antibiotic-treated mice	Increased tubular injury[1]	Osada et al. [9], (2021)
1) *Lactobacillus casei* and *Lactobacillus acidophilus* (4 weeks before IRI) 2) Antibiotics neomycin, vancomycin, metronidazole, penicillin, streptomycin, bacitracin, ciprofloxacin, ceftazidime, gentamicin	Lower macrophage and chemokine stimulation (low F4/80+, CCR2 and CX3CR1, iNOS, and CCL2) as well as macrophage and neutrophil infiltration into kidneys in *L. casei*-supplemented group. Also, increase in nicotinamide levels in kidneys	Increase in *Bacteroidetes* population with higher SCFA-producing bacteria *Alloprevotella* and *PrevotellaceaeNK3B31* (outcomes better in *L. casei* group compared to *L. acidophilus* group)	– Reduced renal injury and Kim-1 levels with *L.casei*[2] – After antibiotics, renal injury lower in *L. casei* group[2]	Zhu et al. [10], (2021)
Lactobacillus salivarius BP121 on days 10–14 after CP	Decrease in MCP-1, kidney IL6, TNFα. Reduced apoptosis, increase in zonulin and occluding proteins. Reduced indoxyl sulfate and p-cresol	Increase in *Lactobacillales* and SCFA	Reduction in tubular injury[2]	Lee et al. [11], (2020)
CP plus *C. butyricum* and *L. reuteri* (10 days after CP) vs. CP only control in Wistar rats	Lower inflammation (KIM-1, F4/80, MPO), fibrosis (collagen IV, fibronectin, a-SMA), blood endotoxin, and indoxyl sulfate	Increase in *Bifidobacterium*, *Ruminococcaceae*, *Ruminiclostridium_9*, and *Oscillibacter*. Decrease in *Escherichia Shigella*	Reduced renal tubular injury and increase in fecal butyric acid in treatment group[2]	Hsiao et al. [12], (2021)

GF, germ-free; IRI, ischemic-reperfusion injury; SCFA, shortchain fatty acids. [1] Increase in renal tubular injury. [2] Reduction in renal tubular injury.

associated with disruption in the intestinal mucosal barrier and activation of immune system-mediated inflammation in various diseases including kidney diseases. Various experimental studies that influence the gut microbiota in ischemic or cisplatin (CP)-induced AKI are detailed below and summarized in Table 1.

The kidney has abundant immune cells. To determine the role of bacteria in generating and maintaining kidney immune cells, experiments were conducted in germ-free (GF) mice by inducing AKI with renal pedicle clamping resulting in ischemic-reperfusion injury (IRI). These studies led to the unexpected findings that GF mice also had abundant immune cells, more NKT cells, and lower IL-4 levels than control mice. Ischemic AKI led to worse tubular injury and functional decline in GF mice compared to ischemia in controls, and the increased susceptibility to injury was normalized after conventionalizing GF mice with feces from normal mice bacteria [3]. Gut microbiota-mediated effects during AKI could be due to properties of SCFAs such as acetate and butyrate that are produced by fermentation end products of certain intestinal microbiota. Acetate supplementation as well as acetate-producing microbiota such as *Bifidobacterium adolescentis* or *Bifidobacterium longum* were shown to reduce $CD11b^+$ and $F4/80^+$ immune cells and improve renal dysfunction and tubular injury after IRI [4]. Possible mechanisms of SCFA actions include activation of G-protein receptors (such as GPR41, GPR43, Olfr78, and GPR109a), histone deacetylase inhibition that alters chromatin remodeling, and preventing decrease in methylation.

In another study on GF mice, renal tubular injury and renal function were worse with lower recovery rates compared with normal mice. Fecal transplant from normal mice attenuated the injury, suggesting renoprotective role of microbiota against ischemic damage. 16S rRNA gene sequencing of mouse feces on days 0, 2, and 10 demonstrated higher microbial diversity with increased abundance of *Lactobacillus, Clostridium*, and *Ruminococcus* and decreased abundance of *Bifidobacterium* and *TM7* microbiota at genus level in the IRI group compared to sham surgery [5]. D-serine was increased in feces, plasma as well as kidneys of IRI mice. D-serine administration reduced tubular injury and was hypothesized to work by attenuating $F4/80^+$ cells and promoting hypoxia-mediated proliferation of tubular epithelial cells [5].

Antibiotics are a clinically translatable approach to modifying gut bacteria during AKI. An AKI prevention antibiotic cocktail of neomycin, metronidazole, ampicillin, and vancomycin decreased injury after IRI [6]. Fecal transplantation of normal mice gut bacteria into GF mice was previously shown to reduce renal injury. However, a study examining GF mice colonized by gut microbiota obtained from standard mice post-AKI led to more severe renal injury [7]. Depletion of gut microbiota with antibiotic combination (neomycin, metronidazole, ampicillin, and vancomycin) conferred renal protection which was associated with reduction in Th17 and Th1 responses along with expansion of regulatory T cells and M2 macrophages. This study provided additional evidence for a bidirectional relationship between AKI and gut dysbiosis mediated via T cells. 16S sequencing of gut microbiota showed a relative increase in *Escherichia* and *Enterobacter* and decrease in *Lactobacillus*, Ruminococcaceae, *Faecalibacterium*, and Lachnospiraceae in the IRI compared to control group. This was associated with lower SCFA and higher endotoxins levels in the IRI group.

A study simultaneously correlating plasma metabolites with gut microbiota and renal function 48 h after IRI found an increase in 31 acetylcarnitines and decrease in 3 amino acids (tyrosine, tryptophan, and proline) [8]. Gut microbiota *Rothia* and *Staphylococcus* species were positively correlated with rise in serum creatinine, while *Prevotella copri, Faecalibacterium prausnitzii*, and *Coprococcus eutactus* were inversely correlated. This suggests that certain gut microbiota may aggravate, while others may ameliorate AKI. In another study, antibiotic-induced gut microbiota depletion using a cocktail of ampicillin, vancomycin, and levofloxacin was associated with reduced concentration of SCFA, renal glucose, and pyruvate levels with more severe tubular injury after IRI [9]. Gut microbiota depletion was hypothesized to increase the vulnerability of kidneys to IRI by possible renal gluconeogenesis-mediated pyruvate depletion. The effect of oral *Lactobacillus casei* administration prior to IRI was also recently studied. Treated mice had less renal damage and better renal function when compared with control mice [10]. A higher proportion of phylum *Bacteroidetes* and SCFA-producing bacteria such as genera *Alloprevotella* and *NK3B31* from family Prevotellaceae were observed in *L. casei*-administered mice. Simultaneous decrease in macrophage stimulation such as F4/80+ and chemokines CCR2 and CX3CR1 in *L. casei*-administered mice suggested it may have been protected by immune regulation of macrophages.

Similar beneficial effects of SCFA-producing bacteria have been observed in CP-induced AKI in two separate studies. One study supplemented *Lactobacillus salivarius BP121* in CP-induced AKI in rats and noted prevention of AKI associated with decrease in inflammation, oxidative stress, and uremic toxins such as indoxyl sulfate and

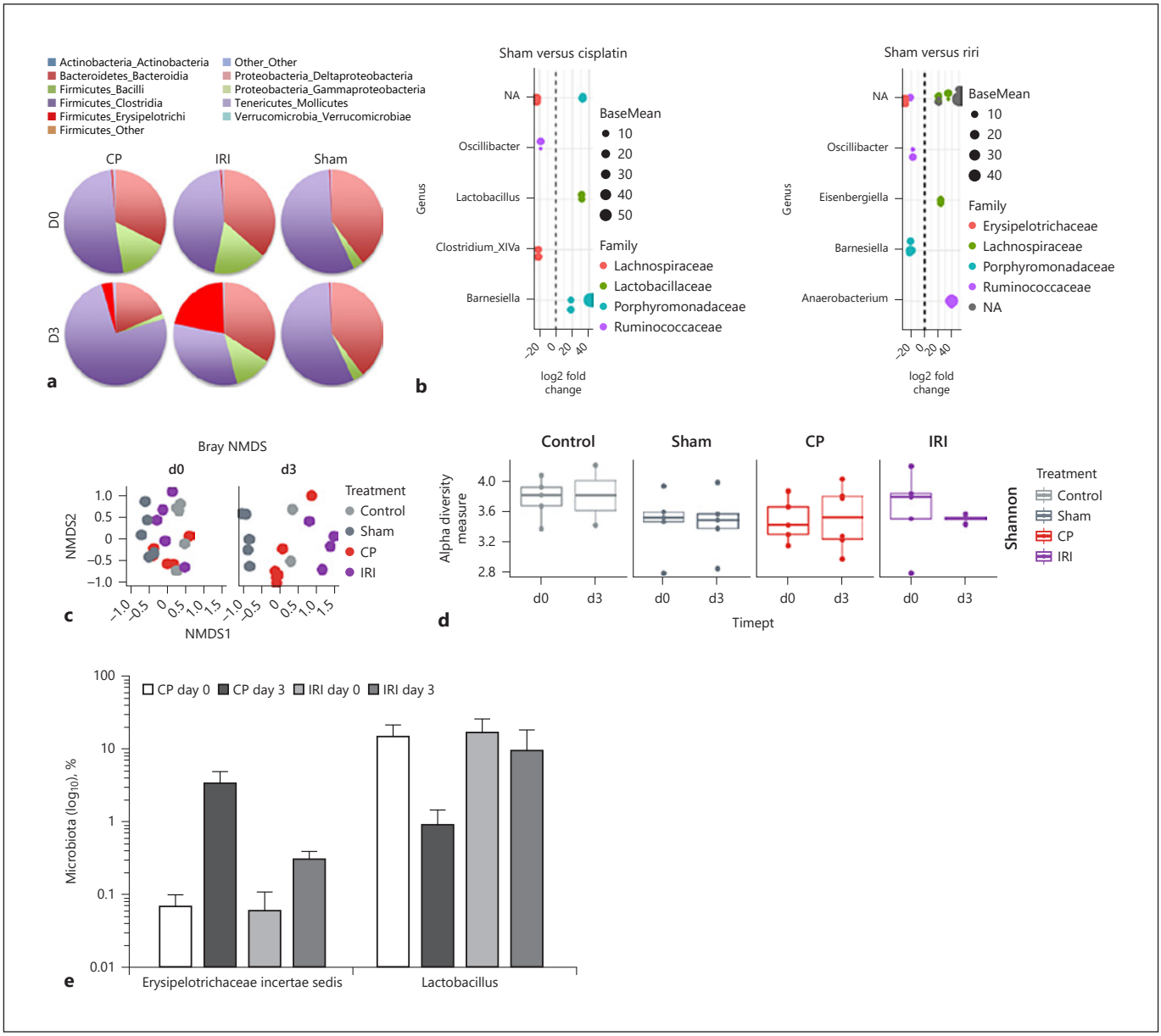

Fig. 1. Renal ischemia-reperfusion injury (IRI) and cisplatin treatment change gut microbial populations. Under an approved animal protocol, AKI was induced in male, 8- to 10-week-old, C57BL/6 mice by 30 min of bilateral IRI or 30 mg/kg cisplatin (CP) injection. The gut microbiota was then studied at baseline (D0) and 72 h (D3) post-AKI using 16S sequencing. **a** IRI and CP affected relative abundance of bacterial species belonging to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Tenericutes*, and *Verrucomicrobia*. **b** The vertical dot plots represent differential abundance testing between sham and CP mice or sham and IRI mice (*x* axis, fold change; size, base mean), showing distinct alterations in microbial populations at the family level. The genera identified on the *y* axis are those that were affected by AKI, using negative binomial testing. There were no significant operational taxonomic units with species-level information. **c**, **d** Dimensional analysis by Bray non-metric multidimensional scaling (NMDS) and *α*-diversity analysis using the Shannon index suggests the microbiome differentiates itself over time, depending on treatment. **e** Percentage change in *E. incertae sedis* and *Lactobacillus* populations after IRI and CP-induced AKI. Timept, time point. NA, not available. Reprinted with permission from "Gut Microbiota-Immune System Interactions during Acute Kidney Injury" by Sanjeev Noel and Hamid Rabb, Kidney360, 2021;2(3):529. Copyright 2020 by the American Society of Nephrology.

p-cresol sulfate [11]. There was an increase in *Lactobacillus* species and SCFA concentration in feces associated with reduction in tight junction protein damage suggesting that the anti-inflammatory effect of this bacteria was mediated by reducing intestinal permeability. Another study supplemented *Lactobacillus reuteri* and *Clostridium butyricum* in CP-treated rats and observed a significant decrease in blood endotoxin and uremic toxin indoxyl sulfate levels which was also associated with decrease in renal inflammation and injury [12]. Gut microbiota changes at genus level were significant for increase in *Bifidobacterium,* Ruminococcaceae*, Ruminiclostridium_9*, and *Oscillibacter* and decrease in *Escherichia Shigella* genera. The findings from these studies indicated that inflammation and renal injury in CP-induced AKI are mediated by gut dysbiosis which is alleviated by butyrate (SCFA)-producing bacteria such as *L. Salivarius*, *L. reuteri*, and *C. butyricum.*

A key component in studies modifying the gut microbiome during AKI is to accurately measure the bacteria in the gut. Given that ischemic AKI could have different effects on the stool microbiome from nephrotoxic AKI, this was evaluated in a mouse model (Fig. 1) [13]. Ischemic AKI at 72 h led to significant changes in families Erysipelotrichaceae, Lachnospiraceae, Porphyromonadaceae, and Ruminococcaceae. At the genus level, significant changes were observed in *Oscillibacter*, *Eisenbergiella*, and *Barnesiella* genera. In contrast, CP-induced AKI led to significant changes in families Lachnospiraceae, Lactobacillaceae, Porphyromonadaceae, and Ruminococcaceae and genera *Oscillibacter, Lactobacillus, Clostridium*, and *Barnesiella*. On further analysis, a significant increase in the proportion of Erysipelotrichaceae *incertae sedis* was observed in both IRI ($p = 0.03$) and CP ($p = 0.007$) groups at 72 h post renal injury when compared to baseline. Conversely, the proportion of *Lactobacillus* decreased significantly ($p = 0.02$) in the CP treatment group at 72 h post renal injury when compared to baseline.

In summary, gut microbiota play an important role in mediating experimental AKI. Current evidence supports a beneficial effect of certain SCFA- and D-serine-producing bacteria in reducing kidney damage and enhancing recovery after ischemic AKI. Given the novelty of this field of research with limited studies and variations in methodology of testing, future studies using consistent testing methods are needed. Measuring precise changes in individual gut microbiota with specific antibiotic and probiotic interventions and mechanistic studies on SCFA and inflammatory cells/molecules is required.

Conclusion

The studies summarized above demonstrate a direct pathophysiologic role of gut microbiota in AKI. The different courses of AKI found in GF mice studies corrected by fecal transplants, as well as interventions modifying AKI in conventional rodents have demonstrated a key role of microbiome in AKI using complementary approaches. A key question in the field of microbiome and AKI is which antibiotic combination is optimal for prevention and which can accelerate the repair of AKI. Other approaches such as probiotics, prebiotics, modified SCFAs and other creative solutions could be used. In addition, there are other microbiome sources besides the gut, such as skin, nares, and even blood and urine that have not been studied in AKI. Finally, human studies on microbiome and AKI are needed to evaluate if similar changes are going on as in experimental models, and the best ways to intervene to improve AKI outcomes.

Statement of Ethics

Review article, not applicable.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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None.

Author Contributions

Neal Shah performed literature review and drafted the manuscript. Hamid Rabb edited and finalized the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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