KI REPORTS
KIReports.org

Effects of Medications on the *in vitro* Growth of Gut Bacteria Associated With Kidney Stones

Jing Bi Karchin[1], Dylan Curry[2], Elliot S. Friedman[2], Michelle Denburg[3,4,5] and Gregory E. Tasian[1,5,6]

[1]Department of Surgery, Division of Urology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; [2]Division of Gastroenterology and Hepatology, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA; [3]Division of Nephrology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; [4]Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA; [5]Department of Biostatistics, Epidemiology, and Informatics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA; and [6]Department of Surgery, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence: Gregory E. Tasian, Children's Hospital of Philadelphia, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. E-mail: tasiang@chop.edu

Received 28 November 2023; revised 9 January 2024; accepted 5 February 2024; published online 9 February 2024

Kidney Int Rep (2024) **9,** 1528–1530; https://doi.org/10.1016/j.ekir.2024.02.005
KEYWORDS: bacteria; kidney stones; xenobiotics

INTRODUCTION

Kidney stone disease (nephrolithiasis) is a disorder of mineral metabolism that is punctuated by painful, episodic events. The prevalence of kidney stone disease has doubled in 30 years with the greatest increases in incidence observed in adolescents and young women.[1,2] Previous studies demonstrated that antibiotics are associated with kidney stone disease[3] and that the gut microbiome and metabolome of children and adolescents with calcium kidney stone disease is altered compared to healthy controls.[4] In particular, bacterial taxa that degrade oxalate and produce butyrate were less abundant among stone formers, suggesting that these bacterial taxa may be lithoprotective. The impact of medications on these taxa is unknown. Here, we determine the effect of commonly prescribed medications that have been associated with kidney stones[3,5,6] on the growth of selected oxalate-degrading and butyrate-producing bacterial taxa *in vitro*.

RESULTS

Ciprofloxacin had a dose-dependent inhibitory effect on the growth of *Oxalobacter formingenes*, *Enterococcus faecalis, Faecalibacterium prausnitzii,* and *Ruminococcus bromii*, and had a mild inhibitory effect on *Lactobacillus gasseri* at all concentrations. At the lowest dose, ciprofloxacin enhanced growth of *F prausnitzii*. Similarly, omeprazole inhibited the growth of *Bifidobacterium animalis* in a dose-dependent manner, with the lowest dose enhancing growth. Omeprazole had a neutral to positive effect on the growth of *L gasseri*. Famotidine had inhibitory or neutral effects on all bacteria except for *L gasseri*, for which growth was increased in a dose-dependent manner. Celecoxib had neutral or dose-dependent inhibitory effects on all bacterial taxa, except at 1 μg/ml, it enhanced growth of *F prausnitzii*. Similarly, simvastatin had dose-dependent inhibitory effects on all bacterial taxa except for mild increases in bacterial growth at 1 μg/ml for *B animalis, E Faecalis*, and *F prausnitzii*. In Figure 1, we present the dose-response effect of the medications on all bacterial taxa.

DISCUSSION

The human gastrointestinal tract is a complicated environment in which the microbiome has an important role in the maintenance of health and susceptibility to disease. Gut bacteria break down medications and produce downstream metabolites that impact the local gut environment and could have downstream effects on other organ systems. In this study, we investigated the effects of several commonly used medications that have been associated with kidney stones and determined their effects on the growth of different bacteria that have the capacity to degrade oxalate or produce butyrate. *B animalis, O formingenes, E faecalis, L gasseri, and F prausnitzii* are capable of

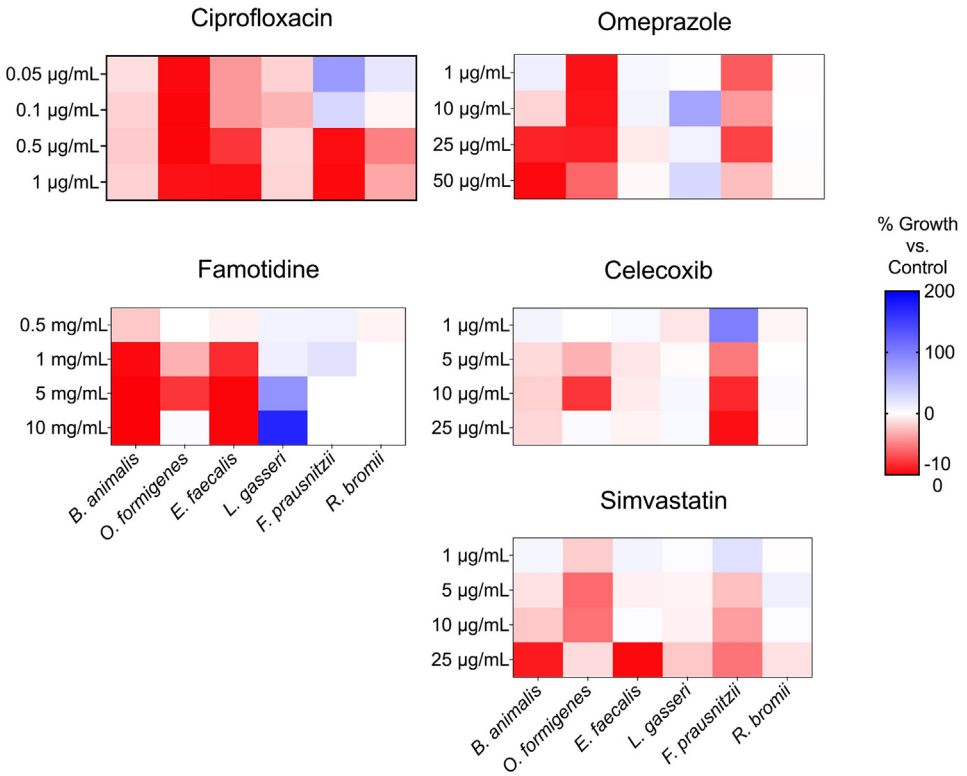

Figure 1. Differential effects of common medications on the growth of gut bacterial taxa. *B animalis, Bifidobacterium animalis*; *E faecalis, Enterococcus faecalis*; *F prausnitzii, Faecalibacterium prausnitzii*, *L gasseri, Lactobacillus gasseri*; *O formingenes, Oxalobacter formingenes*, *R bromii, Ruminococcus bromii*. Dosages of the medications are indicated on the Y-axis. The results were color-coded to reflect percentage of growth versus control. Blue: promotes growth. Red: inhibits growth.

degrading oxalate (the first 4 bacteria listed in Figure 1), whereas *F prausnitzii* and *R bromii* produce butyrate (the last 2 bacteria listed in Figure 1). Butyrate is a short-chain fatty acid and is a mediator of inflammation, protects against renal ischemic damage, helps maintain the gut mucosal barrier, and regulates intestinal expression of SLC26 oxalate transporters.[7] Loss of bacteria that degrade oxalate and produce butyrate may act synergistically to increase intestinal absorption and urinary excretion of oxalate to increase the risk of kidney stone formation.[4] Our results showed that different concentrations of commonly prescribed medications have differential effects on the growth of these bacterial taxa, with most demonstrating greater inhibitory effects at higher doses.

Ciprofloxacin is a fluoroquinolone antibiotic commonly used to treat urinary tract infections. Our group previously demonstrated that fluoroquinolones were 1 of 5 antibiotic classes associated with an increased risk of kidney stone disease.[3] In this study, we detected a broad inhibitory effect of ciprofloxacin on all tested bacteria, which supports the epidemiologic association between fluoroquinolones and kidney stones.[3,8] The consistency of this association is uncertain because a recent cohort study found no association between oral antibiotics and kidney stones.[9] The impact of resistance of "antilithogenic" bacteria to antibiotics such as ciprofloxacin on nephrolithiasis risk also remains unclear.

There was greater heterogeneity in the direction of the effect of the other medications by concentration and bacterial taxa. Simvastatin is a statin that decreases cholesterol biosynthesis by inhibiting HMG-CoA reductase. Statins were associated with a 49% decreased risk of nephrolithiasis among 57,232 members of the US military and their family members.[S1] In an electronic health record study of 101,259 adults with hyperlipidemia, those who initiated statins had a lower risk of developing stones, with the protective effect more pronounced among individuals with a history of nephrolithiasis.[S2] Our results show that simvastatin had neutral or inhibitory effects for most doses and for most bacterial taxa. These results suggest that any potential protective effect of simvastatin on stone formation is not mediated by these bacteria.

Similarly, we tested the effect of celecoxib, an anti-inflammatory cyclooxygenase-2 inhibitor that blocks the synthesis of prostaglandins, on the growth of these bacteria because of the association between inflammation and stone formation.[S3] Celecoxib unsurprisingly did not consistently increase the growth of any of these putative antilithogenic bacteria. The gut microbiome is one

component on the causal pathway for kidney stone disease. Medications such as celecoxib that do not impact bacterial growth may exert an effect on kidney stone formation through other mechanisms such as inflammation in the kidney. Future investigation should categorize drugs associated with an increased risk of kidney stone disease into those that directly impact bacterial survival mechanisms and those that do not.

Omeprazole and famotidine are both medications that treat gastro-esophageal reflux disease. Omeprazole is a H+/K+ ATPase proton-pump inhibitor that inhibits acid secretion from gastric parietal cells and is used in the treatment of *Helicobacter pylori* infection.[S4] Famotidine is a H2 receptor antagonist that blocks the uptake of endogenous histamine into the gastric parietal cells to decrease the production of stomach acid. We found that both omeprazole and famotidine increased the growth of *L gasseri*, though weakly. Generally, higher doses of omeprazole and famotidine inhibited the other oxalate degraders and either inhibited or had no effect on the butyrate-producing *Ruminococcus*. These results are consistent with the associations between proton pump inhibitors and H2 antagonists and a dose-dependent increase in kidney stone risk.[S5]

These results provide preliminary evidence that commonly prescribed medications promote or inhibit the growth of bacterial taxa that could degrade oxalate and/or produce butyrate. A limitation of this study is that the experiments were conducted *in vitro* and on single bacterial taxa, which does not represent the complexity of the intestinal microbiome and the interdependence of bacterial taxa. In addition, whereas *B animalis*, *E faecalis*, and *L gasseri* have the capacity to degrade oxalate, they do not do so preferentially and "prefer" other energy sources to oxalate. The exact role of these species on overall oxalate homeostasis remains unknown as does the trigger for when they switch to oxalate degradation. Future experiments should determine the effect of physiologic doses of these medications at durations of exposure that reflect clinical use on communities of bacteria as they exist *in vivo*. Determining these effects in more complex models that better reflect *in vivo* environments would advance our understanding of how the intestinal microbiome might mediate the effect of common exposures on kidney stone formation and would move us closer to developing therapeutics that restore or replace lithoprotective communities of organisms.

DISCLOSURE

GET reports grant support to their institution from the National Institute of Diabetes and Digestive and Kidney Diseases. MD reports grant support to their institution from the National Institute of Diabetes and Digestive and Kidney Diseases and is an Associate Editor of Journal of the American Society of Nephrology. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

This study was supported by NIH R01DK122156. We would also like to acknowledge the Microbial Culture & Metabolomics Core of the PennCHOP Microbiome Program and the Center for Molecular Studies in Digestive and Liver Diseases (NIH P30DK050306). The following reagent was obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project: *Oxalobacter formigenes*, Strain OxCC13, HM-1.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

REFERENCES

1. Hill AJ, Basourakos SP, Lewicki P, et al. Incidence of kidney stones in the United States: the continuous national health and nutrition examination survey. *J Urol*. 2022;207:851–856. https://doi.org/10.1097/JU.0000000000002331
2. Tasian GE, Ross ME, Song L, et al. Annual incidence of nephrolithiasis among children and adults in South Carolina from 1997 to 2012. *Clin J Am Soc Nephrol*. 2016;11:488–496. https://doi.org/10.2215/CJN.07610715
3. Tasian GE, Jemielita T, Goldfarb DS, et al. Oral antibiotic exposure and kidney stone disease. *J Am Soc Nephrol*. 2018;29:1731–1740. https://doi.org/10.1681/ASN.2017111213
4. Denburg MR, Koepsell K, Lee JJ, Gerber J, Bittinger K, Tasian GE. Perturbations of the gut microbiome and metabolome in children with calcium oxalate kidney stone disease. *J Am Soc Nephrol*. 2020;31:1358–1369. https://doi.org/10.1681/ASN.2019101131
5. Sur RL, Masterson JH, Palazzi KL, et al. Impact of statins on nephrolithiasis in hyperlipidemic patients: a 10-year review of an equal access health care system. *Clin Nephrol*. 2013;79:351–355. https://doi.org/10.5414/CN107775
6. Simonov M, Abel EA, Skanderson M, et al. Use of proton pump inhibitors increases risk of incident kidney stones. *Clin Gastroenterol Hepatol*. 2020;19:72–79.e21. https://doi.org/10.1016/j.cgh.2020.02.053
7. Finnie IA, Dwarakanath AD, Taylor BA, Rhodes JM. Colonic mucin synthesis is increased by sodium butyrate. *Gut*. 1995;36:93–99. https://doi.org/10.1136/gut.36.1.93
8. Ferraro PM, Curhan GC, Gambaro G, Taylor EN. Antibiotic use and risk of incident kidney stones in female nurses. *Am J Kidney Dis*. 2019;74:736–741. https://doi.org/10.1053/j.ajkd.2019.06.005
9. Thongprayoon C, Vaughan LE, Barreto EF, et al. Outpatient antibiotic use is not associated with an increased risk of first-time symptomatic kidney stones. *J Am Soc Nephrol*. 2023;34:1399–1408. https://doi.org/10.1681/ASN.0000000000000155