FINAL REPORT

The Utility of *Oxalobacter* in Enhancing Enteric Oxalate Excretion in Primary Hyperoxaluria Type 1

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INTRODUCTION

In Primary Hyperoxaluria Type 1 (PH1), an increased endogenous production of oxalate, due to a deficiency of one liver enzyme alanine-glyoxylate aminotransferase (AGT), results in hyperoxaluria and calcium oxalate kidney stone formation. The constant elevation in urinary oxalate in PH1 patients can ultimately lead to oxalosis, renal failure and death unless early aggressive clinical management is instigated. Although vitamin B_6 administration is effective in reducing hyperoxaluria in a sub-set of PH1 patients, currently, the only known cure for PH1 is a liver or liver-kidney transplant. Clearly, other treatment options for this disease require attention and we are proposing to examine promoting intestinal excretion and degradation of oxalate as a way of managing this disease.

Two key pieces of information have emerged from our recent studies of colonic oxalate transport in rats which have provided the direction for the studies proposed here. First, the large intestine is the primary site for adaptive enteric excretion of oxalate in oxalate-loaded rats and in rats with chronic renal failure. Second, we have shown that the substrate/oxalate-specific microorganism, *Oxalobacter sp.*, which resides exclusively in the large intestine, can lower urinary oxalate excretion by inducing colonic oxalate secretion. Together, these findings would suggest that robust intestinal colonization with *Oxalobacter* in PH1, or dietary supplementation with *Oxalobacter* cells or products could significantly lower the body burden of oxalate generated by the AGT-deficient liver. Perhaps it is possible to exploit these mechanisms to reduce the oxalate burden in PH1? Now that we have an animal model of PH1, we have a unique opportunity to directly address these questions and obtain novel information regarding the molecular identity of the oxalate transporters that may be involved in enteric oxalate elimination.

In Aim 1 we tested the hypothesis that adaptive enteric excretion of oxalate occurs in PH1 by changes in oxalate transport across the large intestine. In Aim 2, we tested the hypothesis that colonizing the large intestine of the PH1 mouse model with *Oxalobacter* results in a reduction in urinary oxalate excretion by further enhancing enteric oxalate excretion and luminal oxalate degradation. Understanding oxalate handling in PH1 and the potential role of *Oxalobacter* in modulating the excessive body burden of oxalate in PH1 are essential for the development of potential pharmacological therapies.

RESULTS

Oxalobacter Colonization of Mice Promotes Enteric Oxalate Secretion

The question we posed was whether colonization of a mouse model of PH1 (AGXT) would lead to changes in intestinal oxalate handling and reduced urinary oxalate excretion. Mice were colonized with a wild rat strain of *Oxalobacter* (OxWR) and unidirectional fluxes of oxalate were measured using standard Ussing chamber techniques for a period of 45 min. Fluxes were determined across three segments of the large intestine and urinary oxalate excretion was measured before and after the colonization procedure. AGXT mice that were not colonized were included in this study as appropriate controls. In summary, colonization resulted in alterations in oxalate fluxes in two of the three segments examined (see **Figure 1 a-c)**. In the non-colonized caecum, a net absorptive flux of oxalate was evident and it was reversed to a significant net secretory flux with colonization and, in the distal colon, a basal net secretory flux of oxalate was markedly enhanced with colonization. Interestingly, oxalate fluxes across the proximal colon were not altered following colonization. These results in proximal colonic tissues are consistent with those we have observed in previous rat colonization studies showing oxalate fluxes across this segment are unaffected by the presence of OxWR. It is important to note that we confirmed the presence of *Oxalobacter* activity in each of the 3 segments at the time of the

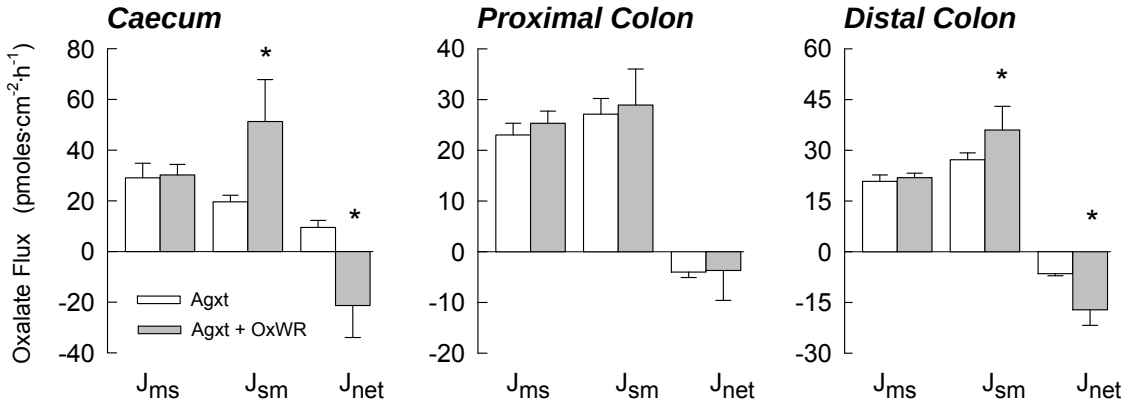

Figure 1. Oxalobacter colonization of AGT knockout mice induces a significant net secretory flux of oxalate across caecum and distal colon. N = 4 Agxt and 9 Agxt + OxWR. An asterisk indicates a significant difference between treatments ($P \leq 0.05$).

flux studies.

Colonization was also associated with a reduction in urinary oxalate excretion. As shown in **Figure 2**, AGXT mice on a C57BL/6 background are hyperoxaluric compared to control C57BL/6 mice. We observed a further increase in hyperoxaluria after the AGXT KO mice were placed on the oxalate-supplemented diet necessary for the colonization procedure. The results in **Figure 2** also show a 50% lower excretion of urinary oxalate in AGXT mice that were colonized compared to AGXT mice not colonized and fed the same oxalate-supplemented diet. Importantly, these values are within the normal limits for urinary oxalate excretion in C57BL/6 mice fed a standard diet. This new information on segment-specific effects of OxWR in AGXT mice is intriguing and demonstrates that the entire large intestine of colonized AGT KO mice functions in an oxalate secretory mode that is associated with a normalization of urinary oxalate excretion in otherwise hyperoxaluric animals.

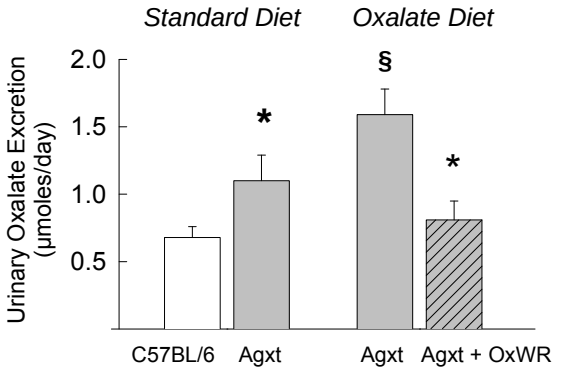

Figure 2. On a standard diet, Agxt mice (n = 11) are hyperoxaluric compared to C57BL/6 mice (n = 18). On an oxalate diet, colonized Agxt mice (Agxt + OxWR) have normal oxalate excretion compared to non-colonized mice (n = 5). An asterisk indicates a significant difference ($P \leq 0.05$) between groups fed the same diet while a § indicates a significant difference between Agxt groups fed different diets.

The effects of colonization were also examined in wild type (WT, C57BL/6) mice in a separate experimental series. The results of the transport studies are presented in **Figure 3 a-c** and illustrate a similar pattern when compared to the results of the AGXT series described above. Again, of the three segments examined, the non-colonized caecum supported a net absorptive flux of oxalate which was reversed to net secretion following colonization. Similarly, both colonic segments exhibited net secretion of oxalate in non-colonized mice and colonization significantly enhanced this secretory flux in the distal colon. While oxalate fluxes across the proximal colon were not altered by *Oxalobacter* colonization of WT or AGXT mice, it was clear that the magnitude of J_{sm}^{Ox} and J_{net}^{Ox} in proximal colon was greater in WT than in AGXT mice whether the mice were colonized or not colonized. As shown in **Figure 4**, urinary oxalate excretion in WT mice fed the oxalate-supplemented diet was almost 4-fold higher than in WT fed the standard diet and this was reduced > 80% by *Oxalobacter* colonization

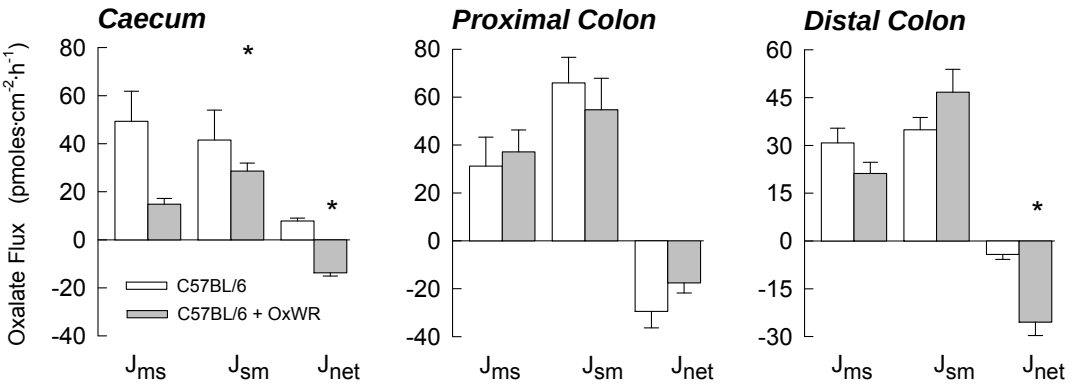

Figure 3. Oxalobacter colonization of C57BL/6 wild type mice induces a significant net secretory flux of oxalate across caecum and distal colon. N = 8 C57BL/6 and 10 C57BL/6 + OxWR. An asterisk indicates a significant difference between treatments ($P \leq 0.05$).

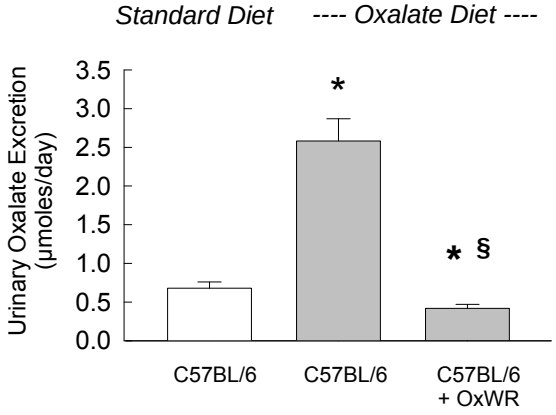

Figure 4. On an oxalate diet, C57BL/6 wild type mice exhibit hyperoxaluria that is abolished following colonization (C57BL/6 + OxWR) . An asterisk indicates a significant difference ($P \leq 0.05$) between the groups indicated and C57BL/6 mice fed the standard chow while a § indicates a significant difference between C57BL/6 + OXWR and non-colonized C57BL/6 on the same diet.

Agxt urinary phenotype

The hyperoxaluric phenotype reported for Agxt mice was confirmed in the present study. Agxt mice of either sex in the pure C57BL/6 background do not form kidney stones and they do not show any other pathology. Baseline urinary excretion values for oxalate, calcium, and creatinine compiled for the WT/Agxt mice used in different experimental series in this study are tabulated in Table 1. We observed that basal urinary oxalate excretion in Agxt was ~2.5-fold higher than in WT fed the same standard diet. We also found that urinary calcium excretion was significantly higher (> 60%) in Agxt mice when compared to WT. It is notable, however, that this hypercalciuria was eliminated when Agxt mice were fed a diet that is low in calcium (0.5%) compared to the standard chow (1% Ca^{2+}). In two of the experimental series below, urinary calcium excretion in Agxt mice fed a low calcium diet (0.81 ± 0.07 µmol/24 h, n=8 pools) was comparable to that of WT mice (0.83 ± 0.09 µmol/24 h, n=9 pools) fed the same diet suggesting the hypercalciuric phenotype of Agxt mice has a large dietary component. Finally, a significant (50%) elevation in serum oxalate concentration was detected in Agxt mice compared to WT.

Table 1. Comparison of selected urinary parameters and plasma oxalate in WT and Agxt mice fed standard mouse chow containing 1% calcium.

	WT	Agxt
Urine Volume ml/24 h	2.46 ± 0.22	3.03 ± 0.24
Urinary oxalate excretion	0.54 ± 0.06	1.30 ±0.15*
Urinary calcium excretion	1.65 ± 0.18	2.66 ± 0.36*
Urinary creatinine excretion	3.80 ± 0.29	4.28 ± 0.20
Ox to Cr excretion ratio	0.08 ± 0.01	0.26 ± 0.03*
Ca^{2+} to Cr excretion ratio	0.35 ± 0.04	0.63 ± 0.07*
Plasma oxalate	20.7 ± 3.9	40.7 ± 4.4*

An asterisk denotes a significant difference, $p \leq 0.05$.

Colonization of AGT-deficient mice reduces serum and urinary Ox^{2-} and promotes enteric Ox^{2-} secretion

Urinary oxalate excretion in Agxt mice was shown to increase further (~ 45%) when these mice were fed the oxalate-supplemented diet necessary for initiating and sustaining *Oxalobacter* colonization. However, when Agxt mice are colonized with *Oxalobacter*, urinary oxalate excretion is reduced to within normal limits seen in WT mice fed a standard diet. The significant difference in urinary oxalate excretion between the two Agxt groups, colonized vs non-colonized, at the time of flux studies appears to be a consequence of the activity of *Oxalobacter* in the large intestine. In addition, colonization of Agxt results in a normalization of serum oxalate concentrations from 40.7 ± 4.4 µM (n=6 serum pools from non-colonized mice) to 20.3 ± 2.6 µM, (n=7 serum pools from colonized mice) revealing a 50% reduction in circulating

oxalate concentrations. Colonized and non-colonized Agxt mice fed a 1.5% oxalate / 0.5% calcium diet exhibited comparable urinary excretion rates for both creatinine (4.23 ± 0.24 µmol/24 h in Agxt, and 5.13 ± 0.64 µmol/24 h in Agxt + OxWR, n=5 and 6 pools, respectively) and calcium (0.89 ± 0.03 µmol/24 h in Agxt, and 0.90 ± 0.09 µmol/24 h in Agxt + OxWR, n=5 and 6 pools, respectively) in the collections obtained immediately before conducting the flux studies. As noted in the previous section, the hypercalciuric phenotype of Agxt mice was eliminated when these mice were provided a diet containing 0.5% calcium. It is also clear that ~ 50% of basal urinary calcium excretion in WT is of dietary origin.

In conclusion, we have demonstrated that segment-specific effects of *Oxalobacter* on intestinal oxalate transport in a PH1 mouse model is associated with a normalization of serum oxalate and urinary oxalate excretion in otherwise hyperoxalemic and hyperoxaluric animals. Whether *Oxalobacter* or products of *Oxalobacter* can be used therapeutically to treat PH patients as well as influence oxalate stone formation in other patient populations warrants long-term investigations.

PUBLICATION

The results of these studies that were supported by OHF were published in the American Journal of Physiology in 2011.

Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of Primary Hyperoxaluria following intestinal colonization with *Oxalobacter.*

Marguerite Hatch[1], Altin Gyjmishka[1], Eduardo C. Salido[2], Milton J. Allison[3], and Robert W. Freel.[1]

American Journal of Physiology (Gastrointestinal & Liver), 2011: 300, G461.

The manuscript can be accessed at:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21163900.