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INVITED REVIEW

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# A new animal model of hyperoxaluria and nephrolithiasis in rats with small bowel resection

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**Abstract** An association between small bowel resection and stone disease has been noted, which is primarily due to increased gut oxalate absorption and resulting excretion by the kidney. In order to better understand the factors affecting both oxalate absorption and renal excretion, and the resulting renal lesions, we have developed a rodent model of small bowel resection and hyperoxaluria. Using this model, we have studied the renal histology in animals with hyperoxaluria over time spans from 2 weeks to 7 months. The initial lesion appears to be crystal formation along the brush border of the proximal tubule, with eventual crystal deposition in collecting ducts and papillary interstitium, and eventual tubule obstruction, interstitial inflammation and fibrosis. Crystal formation appears to dissociate from urinary supersaturation. We hypothesize that oxalate transporters in the proximal tubule may increase local saturations, leading to crystal formation at this site initially. Further studies are required to better characterize the causes and consequences of hyperoxaluria in this animal model.

**Keywords** Kidney calculi · Hyperoxaluria · Calcium oxalate · Small bowel resection

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The association between small bowel resection or bypass and increased urinary excretion of oxalate was first noted by Lynwood Smith and associates [1]. Further work has found that the incidence of stones is increased about threefold after such surgery, and stones are mainly composed of calcium oxalate (CaOx) [2]. The source of the oxalate is increased absorption of dietary oxalate, and the presence of colon is important [3], however, the mechanisms of increased absorption are not fully understood. The degree of hyperoxaluria is influenced by the length of bowel resected, and the amount of fecal fat, as well as dietary intake of oxalate. However, hyperoxaluria is not the sole risk factor for stones in such patients, who frequently also exhibit hypocitraturia, hypomagnesuria, and low urine volumes. Thus, as nicely demonstrated by Smith in a careful study comparing patients with enteric hyperoxaluria (EH) and primary hyperoxaluria (PH), although the urinary oxalate excretion in patient with EH may be only half that in patients with PH, the concomitant presence of these additional risk factors may result in a final urinary supersaturation with respect to CaOx that is significantly higher in the EH subjects [4].

Another factor that influences absorption of oxalate in food, and therefore urinary oxalate excretion, is calcium intake [5, 6]. Dietary calcium can bind oxalate in the gut and lower concentrations of ionic oxalate available for absorption, so that low calcium diets can lead to increased oxalate absorption and urinary excretion. Von Unruh et al. have demonstrated the relationship between dietary calcium and the percent of ingested oxalate absorbed, over the range of normal calcium intakes, with a moderate oxalate intake [7]. In normal subjects, oxalate absorption ranges from 2–20% of dietary intake [8]; however, in patients with small bowel resection and hyperoxaluria, absorption can be as high as 35–50% of ingested oxalate; increased calcium intake can also diminish oxalate absorption in these individuals [2].

In order to gain a better understanding of the factors influencing hyperoxaluria and CaOx nephrolithiasis in

the setting of small bowel resection, we have developed a rodent model of small bowel resection with hyperoxaluria [9]. In this model, hyperoxaluria results from the absorption of dietary oxalate, with resultant renal excretion. This may better mimic the situation in human stone formation, particularly stones associated with altered GI absorption.

Male Sprague-Dawley rats weighing 150–180 g, and acclimated to a room temperature of 25°C with a 12-h light/12-h dark cycle, were fed a standard diet until 24 h prior to surgery; they had free access to water. Resected rats had removal of the distal 40–45 cm of small intestine measured from the ileocecal valve, followed by re-anastomosis of the intestine; control rats underwent transection of the distal ileum without any intestinal excision, followed by re-anastomosis. Rats were given standard rat chow (1.01% Ca, 5.7% lipid) beginning 8 h after surgery, until they had regained their pre-operative weight, usually 7–10 days. They were then placed in individual cages and fed 15 g of either the control chow or the experimental diet (1% Na oxalate, 0.02% Ca, 18% lipids, referred to as high oxalate diet), until the date of sacrifice, with free access to water. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Metabolic cages were used for the collection of 24-h urines, which were collected in thymol, with or without HCl. Urines were analyzed for volume, calcium, oxalate, citrate, creatinine, phosphorus, sodium, potassium, magnesium, ammonia, sulfate, uric acid and pH.

At the time of sacrifice, animals were anesthetized and kidneys removed, with fixation either by immersion in formalin, or perfusion with 5% paraformaldehyde. Kidneys were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Calcium-containing salts were detected with the Yasue method [10]. Micro-FTIR analysis was done on selected sections to confirm the mineral composition of visualized crystals, as previously described [11].

The 24 h urines confirmed that animals on the high oxalate diet became hyperoxaluric, compared with animals on normal chow. Both resected and transected animals had increased urine oxalate excretion on the high oxalate diet but excretion was significantly higher in the resected animals [9]; urine citrate was also significantly lower in the resected rats.

In our initial studies, renal histology was compared in resected and transected rats after 4, 5, 6, and 7 months on the high oxalate diet [9]. While 12 of the 15 resected rats made kidney stones, none of the transected rats did so, and only resected rats had calcifications in the kidney. Crystals filled the lumens of collecting ducts, causing obstruction and inflammation, as well as tubular ectasia which progressed from the medulla to the cortex. Cortical crystal deposition appeared to be localized to sites of tissue injury. Collecting ducts filled with crystals had epithelial cell necrosis and apoptosis. Crystals were also found in the interstitium beneath the papillary surface epithelium, associated with interstitial inflammation and fibrosis. Papillary tip necrosis occurred in the animals with large amounts of interstitial crystal accumulation. Transitional epithelial cell hyperplasia was seen at sites of pelvic crystal accumulation. By infrared spectroscopy, crystalline deposits in the renal pelvis as well as in tubules contained both calcium oxalate and calcium phosphate, as well as some calcium carbonate.

More recent studies have focused on earlier time points in the development of renal crystal deposition in these rats. Hyperoxaluria appears to be more pronounced in this set of animals, perhaps because of a change to a powdered form of the diet, but resected animals continue to excrete significantly more oxalate than transected rats. Serum creatinine, Ca, and electrolytes did not differ between the groups. In preliminary work, we found birefringent crystals at the brush border of proximal tubule cells after 2 weeks on the high oxalate diet. By 4 weeks, crystals were found in both proximal and distal tubule lumens, tubule lining cells contained cytoplasmic vacuoles and there was scattered complete cell loss near crystals. After 8 weeks on a high oxalate diet, crystals were seen in the papilla in a few inner medullary collecting ducts and at the base of the urothelium, along with proliferation of urothelial cells. Among the rats with small bowel resection, we noted that urine oxalate (corrected for creatinine) rose with time in animals without renal tubular crystals, while in rats with crystals it did not rise. No crystals were seen in resected animals on standard chow, although the calculated urinary supersaturation with respect to calcium oxalate was actually higher in these animals compared to the resected rats on a high oxalate diet. The ratio of mean calcium to mean oxalate in the urines (umol/umol) was markedly different, however: 0.027 vs 8.5 (high oxalate diet vs normal chow).

We hypothesize that in the animals on a high oxalate diet, secretion of oxalate into the proximal tubule lumen may produce local supersaturations high enough to cause crystal formation. The crystals may localize to sites of oxalate secretion, and possibly lead to injury to the cells and their transporters, partially limiting oxalate excretion. Several possible anion exchangers that transport oxalate are found in the apical and basolateral membrane of proximal tubules [12]. Similar transporters are also found in the gut, and alterations in their function may play a role in the augmented oxalate absorption seen after bowel resection. Previous studies have suggested a role for such transporters in states of chronic hyperoxaluria due to oxalate ingestion [13], but longer term studies are needed to correlate renal histology with functional information.

The hyperoxaluria in our animals is fostered both by the removal of a portion of the small bowel, and also by the nature of the diet. In addition to containing a large amount of oxalate, the diet is also very low in calcium, which is known to augment gut oxalate absorption. The additional lipid may also play a role; others have shown that adding lipid to the diet can increase oxalate

absorption and excretion [14]. The time course of oxalate excretion in this model may be more similar to that seen in most human stone disease, in which oxalate absorption after meals would be expected to lead to intermittent surges in oxalate traversing the kidney. Prior studies have noted differences in oxalate handling in models of hyperoxaluria induced by feeding oxalate compared to those produced by feeding an oxalate precursor such as ethylene glycol [13].

Further studies are needed to provide information on the role of the bowel transection and the diet, and on the role of oxalate transporters, in the evolution of the lesions seen in the kidney during hyperoxaluria. This may lead to the ability to better modify the course of renal crystal formation and damage due to hyperoxaluria.

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## References

1. Smith LH, Fromm H, Hofmann AF (1972) Acquired hyperoxaluria, nephrolithiasis, and intestinal disease. Description of a syndrome. N Engl J Med 286: 1371
2. Worcester EM (2002) Stones from bowel disease. Endocrinol Metab Clin North Am 31: 979
3. Parks JH, Worcester EM, O'Connor RC, Coe FL (2003) Urine stone risk factors in nephrolithiasis patients with and without bowel disease. Kidney Int 63: 255
4. Smith LH (1992) Hyperoxaluric states. In: Coe FL, Favus MJ (eds) Disorders of bone and mineral metabolism. Raven Press, New York, p 707
5. Lemann JJr, Pleuss JA, Worcester EM, Hornick L, Schrab D, Hoffmann RG (1996) Urinary oxalate excretion increases with body size and decreases with increasing dietary calcium intake among healthy adults. Kidney Int 49: 200
6. Hess B, Jost C, Zipperle L, Takkinen R, Jaeger P (1998) High-calcium intake abolishes hyperoxaluria and reduces urinary crystallization during a 20-fold normal oxalate load in humans. Nephrol Dial Transplant 13: 2241
7. Von Unruh GE, Voss S, Sauerbruch T, Hesse A (2004) Dependence of oxalate absorption on the daily calcium intake. J Am Soc Nephrol 15: 1567
8. Holmes RP, Goodman HO, Assimos DG (2001) Contribution of dietary oxalate to urinary oxalate excretion. Kidney Int 59: 270
9. O'Connor RC, Worcester E, Evan AP, Meehan S, Kuznetsov D, Laven B, Sommer AJ, Bledsoe SB, Parks JH, Coe FL, Gerber GS (2005) Nephrolithiasis and nephrocalcinosis in rats with small bowel resection. Urol Res 33: 105
10. Yasue T (1969) Histochemical identification of calcium oxalate. Acta Histochem Cytochem 2: 83
11. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SM, Shao Y, Sommer A, Paterson R, Kuo R, Grynpas MD (2003) Randall plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. J Clin Invest 111: 607
12. Mount DB, Romero MF (2004) The SLC26 gene family of multifunctional anion exchangers. Pflugers Arch 447: 710
13. Hatch M, Freel RW (2003) Renal and intestinal handling of oxalate following oxalate loading in rats. Am J Nephrol 23: 18
14. Ferraz RR, Tiselius HG, Heilberg IP (2004) Fat malabsorption induced by gastrointestinal lipase inhibitor leads to an increase in urinary oxalate excretion. Kidney Int 66: 676