The Roles and Mechanisms of Intestinal Oxalate Transport in Oxalate Homeostasis

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Summary: The mammalian intestine has an important role in the dynamics of oxalate exchange and thereby is significant in the etiology of calcium oxalate nephrolithiasis. Here we review some of the phenomenologic observations that have led to the conclusion that anion exchangers (antiporters) are important mediators of secondarily active, net oxalate transport along the intestine (both absorptive and secretory). Understanding the mechanisms of transepithelial oxalate transport has been advanced radically in recent years by the identification of the solute-linked carrier (SLC)26 family of anion transporters, which has facilitated the identification of specific proteins mediating individual apical or basolateral oxalate transport pathways. Moreover, identification of specific exchangers has underscored their relative importance to oxalate homeostasis as revealed by using knockout mouse models and has facilitated studies of oxalate transport regulation in heterologous expression systems. Finally, the significance of oxalate degrading bacteria to oxalate homeostasis is considered from basic and applied perspectives.
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Hyperoxaluria is considered to be a major risk factor in calcium oxalate nephrolithiasis, which occurs in about 12% of the population.[1] The importance of the intestine in this renal disease stems from 3 facts. First, because urinary oxalate ultimately is derived from dietary (net intestinal absorption) as well as endogenous (hepatic metabolism) avenues,[2] intestinal oxalate absorptive mechanisms are significant. Second, although the principal route for oxalate excretion is through the kidneys, considerable intestinal excretion (net intestinal secretion) of oxalate occurs in some pathologic conditions, which has adaptive significance.[3,4] Third, certain microorganisms resident in the mammalian gut can degrade oxalate,[5-11] suggesting they also potentially can contribute to the mass balance of oxalate.

In this brief overview of the role of the intestine in oxalate homeostasis we consider some of the phenomenologic aspects of intestinal oxalate transport (handling) that have led to the notion that the bulk of net transcellular oxalate transport, either absorption or secretion, occurs via anion exchangers (antiporters). We then consider the emerging importance of gene families encoding these anion exchangers, especially solute-linked carrier (SLC)26, and how an understanding of these proteins and their segmental and cellular distribution, has led to a better understanding of intestinal oxalate exchange in health and disease. Finally, recent information on the role of oxalate degrading bacteria (*Oxalobacter*) in modulating intestinal oxalate handling is considered. Previous reviews of oxalate handling in intestinal[10,12-14] and renal epithelia[15,16] may be consulted for additional perspectives (eg, animal models simulating oxalate-associated disease states, factors influencing intestinal oxalate absorption).

PHENOMENOLOGIC ASPECTS OF INTESTINAL OXALATE TRANSPORT

The transepithelial, unidirectional flux of any solute (mucosal to serosal or serosal to muco-

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sal) is the sum of parallel flows through paracellular and transcellular pathways. Establishing the relative importance of these 2 avenues is fundamental to understanding oxalate handling in vivo, but no systematic study has been presented to quantitate their relative roles in secretion or absorption. Early models for oxalate absorption in the intestine favored a nonmediated, passive, gradient-driven flux through paracellular pathways.[17] However, it later became apparent that an energy-dependent, net absorption of oxalate could be shown using short-circuited rat distal colon in the absence of a transepithelial oxalate gradient and using physiologic concentrations of oxalate (<2 μmol/L) on both sides of the epithelium.[18] The apparent active, transcellular absorption of oxalate we observed in the rat colon promoted a number of additional studies characterizing the segmental heterogeneity, ion dependencies, inhibitor profiles, and some regulatory aspects of oxalate transport in rat and rabbit intestine that are considered later.

Paracellular Exchange

Before considering the transcellular avenues in detail we would like to re-emphasize that in vivo the relative significance of paracellular (shunt) oxalate transport (in the secretory or absorptive direction) is defined only vaguely. Because flow through this pathway is dependent on the prevailing transepithelial electrochemical potential differences of the oxalate anion, it is anticipated that different segments will support different levels of passive oxalate transport. In this sense, it might be expected that along the early portions of the intestine—where junctional resistance is typically low and luminal oxalate activity is expected to be higher—that the shunt contributes significantly to oxalate absorption. In contrast, the contribution of paracellular pathways to oxalate absorption in more distal segments such as the colon—where luminal oxalate activity might be lower and junctional resistance is much higher—is likely to be less.

The importance of paracellular oxalate absorption is best shown in the pathology of enteric hyperoxaluria associated with the malabsorption of fatty acids and bile salts.[17,19,20] Normally, these luminal solutes are absorbed efficiently in the small intestine. However, in malabsorption syndromes (secondary to disease[21-24] or small-bowel resection[25]) these secretagogues promote increases in the paracellular permeability of the large intestine,[23,26] leading to the passive hyperabsorption of oxalate in proportion to the luminal activity of the oxalate ion.[20]

Transcellular Absorption

Apical uptake

Although the initial studies with isolated rat distal colon clearly indicated net absorption of the oxalate ion in the absence of any electrochemical driving forces,[18] the nature of this active oxalate absorption was not clear. The transcellular absorption of oxalate requires an uptake mechanism at the apical membrane followed by an efflux mechanism across the basolateral membrane of the epithelium. In the course of several studies using isolated, short-circuited segments of rabbit distal colon it became apparent that net oxalate absorption was an energy-dependent process showing many characteristics that were emerging concurrently for active chloride absorption. For example, net oxalate absorption and sodium absorption were inhibited by 100 μmol/L 2-4 dinitrophenol, an uncoupler of oxidative phosphorylation, confirming the energy-dependence of these active transport systems.[27] Also, in the rabbit distal colon the anion exchange inhibitor 4-acetamido-4-isothiocyano-2,-stilbene-2,2′-disulfonic acid applied mucosally (100 μmol/L) inhibited net chloride and oxalate absorption without affecting short-circuit current, primarily by reducing the M to S unidirectional fluxes.[27] Furthermore, the carbonic anhydrase inhibitor acetazolamide significantly reduced net chloride and oxalate absorption in the nominal absence of HCO_3^- in the buffers. From these findings it was concluded that oxalate absorption was a secondarily active process that likely was mediated apically by an anion exchanger (antiporter) that was similar to that mediating chloride absorption. Somewhat later these notions regarding apical oxalate transport were confirmed and extended in studies using brush-

border membrane vesicles (BBMVs) isolated from the rabbit distal ileum.[28,29] From the latter studies it was predicted that there were at least 3 separate anion exchangers in rabbit BBMVs: a Cl^-/HCO_3^- exchanger; a SO_4^{2-}/OH^- exchanger, which also transports oxalate; and an Ox^{2-}/Cl^- exchanger, all of which were reported to be electroneutral. Thus, 20 years ago there was strong empiric support for the notion that net transepithelial oxalate absorption was mediated apically by anion antiporters more commonly regarded as chloride/base exchangers, a fact that presaged current efforts to establish the roles of multifunctional anion exchangers in the SLC26 gene family to oxalate handling.

Basolateral efflux

Concomitant with the recognition of apical antiporters as mediating oxalate uptake in the intestine was the empiric development of the idea that anion exchange also may account for oxalate efflux across the basolateral membrane of absorptive enterocytes. Thus, in rabbit distal colon serosal addition of 1 μmol/L 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) reduced net oxalate absorption by 43% without affecting net chloride absorption, and serosal Na^+ removal decreased net oxalate absorption without affecting net Cl^- absorption.[30] In addition, net oxalate absorption (but not Cl^- absorption) showed a dependence on serosal (but not mucosal) Na^+ as judged by the effects of serosal amiloride (1 mmol/L) and serosal Na^+ replacement.[30] It was concluded that the mechanism(s) mediating oxalate efflux across the basolateral membrane during absorption was an anion exchanger (DIDS-sensitive) mechanistically distinct from that mediating apical uptake. The marked sodium dependence was proposed to result from a coupling of base anion/oxalate exchange with pH regulation via a basolateral Na^+/H^+ exchanger.[30]

Transcellular Secretion

Up until the early 1990s it appeared that the mammalian intestine functioned only in an absorptive mode with respect to oxalate, but it now is clear that net enteric oxalate secretion/excretion also occurs, which may have a significant impact on oxalate homeostasis. Studies using short-circuited tissue preparations from rabbits,[30,31] rats,[4,14] and more recently mice[32,33] have indicated that oxalate handling along the mammalian intestinal tract varies in both magnitude and direction in a segment-specific manner. Invariably, under control conditions, the small intestine and proximal colon show a net secretion of oxalate whereas the distal colon supports net oxalate absorption. Under certain conditions, after the addition of cyclic adenosine monophosphate (cAMP) for example, the distal colon can support a net secretory flux of oxalate.[30] We have proposed that such secretagogue-induced oxalate secretion across this segment is mediated by a furosemide-sensitive basolateral uptake process and passive diffusion of accumulated oxalate through an apical conductive pathway that can be blocked partially by the Cl^- channel blocker 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB).[30,34] We proposed that electrogenic oxalate flux in BBMVs[34] was mediated by an anion channel such as cystic fibrosis transmembrane conductance regulator (CFTR), however, we recently observed that ^{14}C-oxalate efflux from *Xenopus* oocytes expressing human CFTR is the same as water-injected oocytes (unpublished observations). This indicates that oxalate secretion, at least in human beings, may not be mediated by the CFTR chloride channel per se. Recent findings that some of the members of SLC26 are electrogenic[35] and functionally interact with the CFTR gene product in a regulatory manner[36,37] may be relevant in this regard.

THE SLC26 GENE FAMILY AND INTESTINAL OXALATE TRANSPORT

Until recently, phenomenologic approaches described earlier represented the sole experimental basis for our current understanding of intestinal oxalate handling. Unraveling mechanisms was difficult because it was not possible to identify specific transport proteins beyond anion selectivity and inhibitor profiles, which often overlap. This has changed considerably with the identification of the SLC gene superfamily, which encodes for proteins that mediate anion transport.[38-40] Particularly relevant to the current discussion are several structurally related proteins encoded by the

SLC26 gene family that are anion transporters with measurable affinity for oxalate and are expressed in the intestine.[38-40] Oxalate-transporting members of this family that are found in the gastrointestinal tract are as follows: SLC26A1 (sulfate anion transporter 1 [SAT1]), SLC26A2 (diastrophic dysplasia sulfate transporter [DTDST]), SLC26A3 (down-regulated in adenoma [DRA]), SLC26A6 (putative anion transporter 1 [PAT1] or chloride formate exchanger [CFEX]), SLC26A7, and SLC26A9. SAT1 is located in the human small intestine and colon[41] and in postconfluent monolayers of Caco-2 cells,[42] presumably basolaterally (as in the proximal tubule[43]). DTDST is relatively abundant in the human large intestine and less in the small intestine[44] and we have observed SLC26A2 messenger RNA in rat intestine[42] and in confluent Caco-2 monolayers.[45] The affinity of DTDST (SLC26A2) for oxalate has not been defined clearly. In mice[46] and rats,[47] DRA (SLC26A3) is abundant in the apical membrane of colonocytes and less so in the small intestine. In mice, PAT1 (slc26a6) is abundant in the apical membrane of the duodenum and ileum but less so in the colon.[48,49] The isoforms SLC26A7 and SLC26A9 have been detected in gastric mucosa[39]; yet, although the stomach may mediate passive oxalic acid absorption by nonionic diffusion,[10] neither the mucosa nor the resident SLC26 transporters have been implicated in mediated oxalate flux.

Studies in Knockout Mice

The utility/importance of identifiable molecular entities is shown best with the use of targeted disruption of genes encoding oxalate transporters. For example, the examination of oxalate transport across the ileum of slc26a6 null mice made by Freel et al[32] revealed that slc26a6 represents a major apical membrane pathway mediating oxalate efflux rather than influx under normal conditions. The wild-type (WT) mouse ileum supported a small net secretion of oxalate that was sensitive to the mucosal addition of DIDS (200 μmol/L), whereas in the PAT1 knockout (KO) mice there was a significant net absorption of oxalate that was DIDS-insensitive. In addition, these KO mice were hyperoxaluric compared with the WT mice, presumably because of the enhanced net absorption of oxalate. This study also showed that Cl^- is an exchange partner for oxalate efflux across the apical membrane of the mouse ileum and there was no evidence that PAT1-mediated oxalate efflux was regulated by intracellular cAMP. By using an independently generated slc26a6 null mouse model, Jiang et al[33] also observed an increase in duodenal oxalate absorption in their PAT1 KO mouse model compared with WT mice. Interestingly, these KO mice had a high frequency of oxalate bladder stones, unlike the KO animals used by Freel et al,[32] despite a similar degree of hyperoxaluria. This curious and important phenotypic difference between the PAT1 KO models possibly may be explained by the different background strains of the mice used in each study and/or the fact that the KO mice used by Jiang et al[33] were significantly hypercalciuric (urinary Ca^{2+} concentration, 6.73 mmol/L $\pm$ 1.71; $n = 10$) compared with the PAT1 KO model used in our studies (1.49 mmol/L $\pm$ 0.35, $n = 5$, unpublished observations). The study by Jiang et al[33] also provided evidence to suggest that a large contribution of urinary oxalate in their PAT1 KO model was derived from dietary sources; however, they could not completely exclude some renal involvement. It is notable that their results did show reductions in oxalate excretion and serum oxalate in the KO mice fed an oxalate-free diet, but, both still were significantly higher than in WT mice fed a similar diet. We would argue that because both intestinal and renal epithelia are the principal interfaces for exchange of oxalate, and because the PAT1 transporter is expressed in both epithelia, it is reasonable to assume that changes in urinary oxalate excretion result from oxalate transport activity at both of these interfaces.

Because PAT1 appears to mediate apical oxalate efflux (cell to lumen) in the mouse small intestine,[32,33] we have speculated that DRA (slc26a3)[32] may mediate apical oxalate uptake (lumen to cell) in the intestine. If this is correct, we would expect that intestinal oxalate absorption should be reduced in DRA KO mice[50] compared with WT mice and urinary oxalate excretion would decrease in DRA KO mice. The following preliminary results (unpublished observations) we have obtained using DRA het-

erozygotes (HETS) as well as DRA KO mice were consistent with this expectation. In the HETS, significant reductions were observed in the mucosal to serosal flux of oxalate in both the distal ileum (~23% decrease) and distal colon (~50% decrease), whereas urinary oxalate excretion was reduced significantly from 1.70 ± 0.23 μmoles/24 h in WT mice compared with 0.87 ± 0.11 μmoles/24 h in the HETS (n = 9 in each group). In the small number (n = 3) of DRA KO mice we have examined thus far, the mucosal to serosal flux of oxalate in the distal ileum was reduced 45% and urinary oxalate excretion also was lower and comparable with the HETS. Thus, in the absence of DRA, the mouse ileum supports a sizeable net secretory flux of oxalate (~9-fold greater than the WT) primarily owing to decreases in the absorptive component of the flux. Because DRA is not present in the kidney,[50,51] the reduced intestinal absorption of oxalate in these mice most likely contributes to the reduction in urinary oxalate excretion in slc26a3 null mice.

Regulation of Intestinal Oxalate Transport and SLC26 Isoforms

Functionally, it has been shown that colonic oxalate secretion can serve as an extrarenal route for oxalate excretion both in rats with chronic renal failure and in rats challenged by an oxalate load.[4,52-54] The chronic renal failure–stimulated enteric secretion was correlated with a local up-regulation of angiotensin II receptors and was shown to be mediated by serosal angiotensin II type 1 (AT_1) receptor activation.[52,53] These studies also provided evidence for hyperoxalemia-induced oxalate secretory pathways that are independent of angiotensin II regulation and distinct from those secretory pathways previously implicated[54] (ie, cAMP-induced secretion). Serosal epinephrine (50 μmol/L) changes net oxalate transport across the isolated, short-circuited rabbit proximal colon from a secretory to an absorptive mode via increases in the mucosal to serosal oxalate flux and a decrease in the serosal to mucosal flux.[31] These phenomenologic studies clearly show that oxalate transport is affected by neurohormonal effectors, but transporters mediating these changes have not been resolved.

There is little presently known about the regulation of oxalate handling mediated by the SLC26 transporters in the intestine, but this is likely to change considerably. It is expected, however, that because most of these anion exchangers are involved with acid-base regulation of intestinal cells, oxalate flux will be affected to some extent by factors regulating intracellular pH.[10] PAT1 is reported to be the principal Cl^-/HCO_3^- exchanger in the apical membrane of the upper-villous epithelium in mouse duodenum with a smaller contribution by DRA.[48] One of the more novel aspects of HCO_3^- regulation (and possibly oxalate transport, directly or indirectly) is the suggested mechanism of acute regulation via disruption of bicarbonate transport metabolons. For example, it has been proposed[55] that SLC26A6 forms a transport metabolon by complexing with carbonic anhydrase II (CAII). Apparently, because the binding location for CAII on PAT1 is adjacent to the protein kinase C (PKC) phosphorylation site, PKC disruption of CAII binding results in reduced bicarbonate transport rates.[55] The significance of this mechanism to SLC26A6-mediated intestinal oxalate transport is not certain because SLC26A6 shows multiple modes of anion exchange independent of HCO_3^- transport.[38] In this regard, it has been reported[56] that phorbol ester activation of PKC reduces the serosal to mucosal flux of oxalate across the mouse duodenum by about 50%, an effect that was blocked by the PKC-δ inhibitor, rottlerin. Whether PKC altered net oxalate transport by mouse duodenum was not reported. In parallel studies of *Xenopus* oocytes expressing mouse slc26a6, PKC activation also reduced Cl^-/Ox^{2-} exchange and this reduction was considered to be secondary to the endocytotic withdrawal of slc26a6 from the oocyte membrane.[56]

The intriguing observations that the SLC26 transporters may interact with CFTR[36,50,57-59] or modify other transporters[47] afford another emerging regulatory aspect of these anion/base transporters. For example, CFTR up-regulates SLC26A3 and SLC26A6 function in cultured pancreatic duct cells[60] and co-expression of DRA or PAT1 with CFTR in HEK293 cells[36,61]

mutually activates their physiologic functions (Cl^--dependent pH changes and Cl^- current, respectively). In the latter study,[36] it was proposed that the SLC26 transporters and CFTR exist in a complex by binding to scaffolds containing PDZ domains and interactions between the CFTR R-domain and the SLC26 STAS domain results in mutual activation of CFTR and the SLC26 transporters.[36,57] Another indication that SLC26 proteins have a regulatory potential comes from a report that rat slc26a3 does not mediate significant DIDS-sensitive Cl^-/HCO_3^- exchange when expressed in HEK293.[47] It was suggested that if this were true for human DRA, then mutations in slc26a3[62] leading to congenital chloride diarrhea (low pH with high Cl^-) could not be caused solely by defective DRA-mediated Cl^-/HCO_3^- exchange per se.[47] This would imply that slc26a3 may act as a modifier gene for other unidentified transporters.[47]

ROLE OF OXALATE-DEGRADING BACTERIA

Several intestinal bacteria have been reported to degrade oxalate and these include *Eubacterium lentum*,[5] *Enterococcus faecalis*,[6] lactic acid bacteria *Lactobacillus sp.*, *Streptococcus thermophilus*, *Bifidobacterium infantis*,[7,8] and *Oxalobacter* sp.[9,10] More recently, a novel oxalate-degrading member of the *Enterobacteriaceae* (*Providentia rettgeri*) was identified in human fecal samples and, notably, this organism also was reported to have enzymes similar to those of *O formigenes*.[11] The major focus of the following section is on the role of the substrate/oxalate-specific *Oxalobacter* sp. in intestinal handling of oxalate simply because it has received the most attention in this regard by investigators. For earlier reviews of the oxalate-degrading bacteria, see articles by Allison et al,[63] Goldfarb,[64] and Hoppe et al.[65] We also recommend the section on *Oxalobacter* in a previous review as background material for the present discussion.[10]

Studies in Animals

Oxalobacter sp. has been found to be present in the intestines of most wild and domesticated animals that have been tested, however, it is not typically present in laboratory rats.[10,63] The results of all of the studies examining the effects of *Oxalobacter* on oxalate handling in laboratory rats are consistent and it generally is concluded that urinary oxalate can be reduced by oral administration of encapsulated oxalate-degrading enzymes from *Oxalobacter*[66,67] or by administering viable whole *Oxalobacter* cells.[68] Reasonably, this effect has been assumed to be caused by degradation of dietary oxalate by the bacterial enzymes produced in the luminal compartment. Certainly, a favorable transepithelial gradient across the intestine will promote the passive movement of oxalate from the blood if luminal oxalate concentrations are maintained at a lower level by *Oxalobacter* enzyme activity, and this may be particularly important in severe hyperoxalemic conditions such as primary hyperoxaluria and enteric hyperoxaluria. We have proposed that, in addition to degrading dietary sources of oxalate, *Oxalobacter* may be able to derive oxalate from systemic sources by initiating or enhancing active secretion of endogenous oxalate.[67] Subsequently, by using various approaches, we were able to show that *O formigenes* can modulate intestinal oxalate transport by inducing colonic oxalate secretion[67] and a positive consequence of this bacterial host cell interaction is a significant reduction in urinary oxalate excretion owing to this enteric oxalate shunt.[67] The latter study also was the first to show that endogenously derived oxalate can sustain *Oxalobacter* colonization. In the next few years, it is anticipated that there will be advances in our understanding of the mechanistic basis for bacterial cell modulation of intestinal oxalate handling because this is fundamental to future efforts in identifying which strains of bacteria and/or bacterial products will be effective in the treatment of hyperoxaluria and calcium oxalate stone disease.

An interesting addendum to this section concerns very recent communications[69,70] showing that a highly specific oxalate-degrading enzyme, formulated as cross-linked enzyme crystals (ALTU-237), significantly reduces hyperoxaluria in a knockout mouse model simulating primary hyperoxaluria, type 1. ALTU-237, administered orally for a month at a dose of 80 mg/d, reduced urinary oxalate by 50% and there was a total

prevention of nephrocalcinosis, renal failure, and death compared with the controls.[70] Although the enzyme was not identified in these studies for proprietary reasons, the efficacy of ALTU-237 in an animal model is impressive and underscores the potential for novel formulations of oxalate-degrading enzymes.

Studies in Human Beings

Based on the results of several studies in human beings, it now appears reasonable to consider the lack of intestinal *Oxalobacter* activity as a risk factor for hyperoxaluria and stones but not necessarily as a direct cause of stone disease.[66,71-76] In fact, other intestinal oxalate-degrading bacteria may be important in this regard also but, as yet, there is no correlative data available for these other known/unknown microorganisms. Several studies have shown that stone-forming patients who lack *Oxalobacter* have significantly higher urinary oxalate excretion compared with patients colonized with the bacteria.[71-74] Additional support for the notion that *Oxalobacter* may confer some protection against stone disease was suggested by other studies[66,75,76] showing a positive correlation between the number of stone episodes and the lack of intestinal *Oxalobacter* activity. More importantly, perhaps, is the concept that supplemental supplies of the oxalate-degrading bacteria or their enzyme products potentially can be used as a treatment to reduce hyperoxaluria and stone disease. In 2002, it was shown for the first time that urinary oxalate was reduced after the administration of a single oral dose of *O formigenes* in 4 human subjects ingesting an oxalate load.[77] More recently, oral administration of *Oxalobacter* was tested in a clinical setting involving several groups of primary hyperoxaluria patients.[65,78-80] The patients were treated with 2 forms of *O formigenes*, either a frozen cell paste or enteric-coated capsules containing the bacteria, and significant reductions in urinary oxalate were observed during treatment. Other positive outcomes, in some but not in all of these patients, included reductions in plasma oxalate concentrations and amelioration in the clinical signs of systemic oxalosis. The results of these human studies are consistent with the animal studies in that *Oxalobacte*r can degrade intraluminal, dietary-derived oxalate available in the intestinal lumen, thereby reducing its absorption. Clearly, the results of these studies are encouraging and support the concept that supplemental supplies of oxalate-degrading bacteria or their enzyme products potentially can be used as a treatment to reduce hyperoxaluria. However, more clinical studies and trials are warranted and future developments in this field must include rigorous double-blind, placebo-controlled trials using probiotics/bacterial products/oxalate-degrading enzymes along with a significant contribution from basic science addressing the mechanism of action.

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