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The role of intestinal oxalate transport in hyperoxaluria and the formation of kidney stones in animals and man

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Abstract

The intestine exerts a considerable influence over urinary oxalate in two ways, through the absorption of dietary oxalate and by serving as an adaptive extra-renal pathway for elimination of this waste metabolite. Knowledge of the mechanisms responsible for oxalate absorption and secretion by the intestine therefore have significant implications for understanding the etiology of hyperoxaluria, as well as offering potential targets for future treatment strategies for calcium oxalate kidney stone disease. In this review, we present the recent developments and advances in this area over the past 10 years, and put to the test some of the new ideas that have emerged during this time, using human and mouse models. A key focus for our discussion are the membrane-bound anion exchangers, belonging to the SLC26 gene family, some of which have been shown to participate in transcellular oxalate absorption and secretion. This has offered the opportunity to not only examine the roles of these specific transporters, revealing their importance to oxalate homeostasis, but to also probe the relative contributions made by the active transcellular and passive paracellular components of oxalate transport across the intestine. We also discuss some of the various physiological stimuli and signaling pathways which have been suggested to participate in the adaptation and regulation of intestinal oxalate transport. Finally, we offer an update on research into *Oxalobacter formigenes*, alongside recent investigations of other oxalate-degrading gut bacteria, in both laboratory animals and humans.

Keywords

Caco-2; Chloride/bicarbonate exchange; DRA; PAT1; Ussing chamber

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures involving animals were performed under protocols approved by the University of Florida Institutional Animal Care and Use Committee (IACUC), in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals”.

Introduction

The vast majority of oxalate in the body is excreted via the kidneys where 90–95% of circulating oxalate is eliminated in the urine [1–3], with the remaining fraction secreted into the intestine. The renal contribution to keeping blood oxalate levels low is therefore obvious, but the value of the intestinal tract to oxalate homeostasis can easily be overlooked. The importance of the intestine stems from its role in dietary oxalate absorption, together with its ability to serve as an extra-renal pathway for excretion. The intestine also houses the gut microbiome. Some of these commensal bacteria are capable of degrading oxalate, thus limiting its absorption and, in the case of *Oxalobacter formigenes*, avidly promoting intestinal oxalate secretion. The influence of the intestine is most evident, and indeed clinically relevant, in cases of disease. Gastrointestinal (GI) disorders (e.g., inflammatory bowel disease), and/or surgery (e.g., gastric bypass), can lead to excessive oxalate absorption and secondary enteric hyperoxaluria [4]. In chronic renal failure, where urinary oxalate clearance is limited, compensatory oxalate secretion by the large intestine is triggered to increase fecal elimination [3, 5, 6]. A final example are the large amounts of oxalate generated by metabolic overproduction in the liver (the primary hyperoxalurias), where this excess has been successfully targeted via the gut, at least in animal models, through colonization with *Oxalobacter* [7–9].

As a valuable extra-renal pathway for eliminating oxalate, knowing how the intestine transports this anion is essential. Illuminating the mechanisms responsible for absorption and secretion has garnered considerable interest, not only for understanding oxalate homeostasis but also for the development of future therapeutic approaches to tackling hyperoxaluria and kidney stone disease. Realizing this potential demands a fundamental understanding of oxalate transport and how it is regulated. Over the past 35 years, four major discoveries have come to shape our present knowledge. The first came in 1980 with the report of an active component to intestinal oxalate transport [10]. The second was subsequent studies revealing the remarkable adaptive capacity of the intestine, where it could be induced to either actively absorb or secrete oxalate on a net basis in response to various local and systemic stimuli [5, 11–13]. The third came with the isolation and identification of *Oxalobacter* [14, 15], but more specifically, its unique ability to induce active oxalate secretion by the intestine [7–9]. The final key development has been identification of the SLC26 (SoLute Carrier) gene family of anion exchangers and the pivotal roles some of these individual transporters play in oxalate transport by the intestine [16–19]. For more expansive background information on these and other facets of intestinal oxalate transport readers are directed to prior authoritative reviews [20, 21]. The intention of this present review is to provide an update of recent developments and advances that have taken place in the field over the past 10 years.

The pathways and mechanisms for oxalate transport across the intestine

Overview

The transport of oxalate by the intestine can be categorized based on the pathway it takes across the epithelium and the underlying mechanism involved. Broadly speaking, these are ‘paracellular and passive’ and ‘transcellular and active’. The former involves oxalate moving

between the epithelial cells in response to the prevailing transepithelial electrical and concentration gradients acting upon the oxalate anion, and also the properties of the tight junctions. For the transcellular pathway, oxalate moves through the cells and this must be facilitated by membrane-bound transport proteins located within the apical and basolateral membranes (Fig. 1). The absorption and secretion of oxalate occur simultaneously across the intestinal epithelium. The absorptive oxalate flux from the lumen (mucosal) to the blood (serosal), denoted J_{ms}^{Ox}, involves paracellular and transcellular pathways, while in the opposite direction, oxalate secretion (J_{sm}^{Ox}) is presently understood to be largely transcellular, although the paracellular route may be significant in cases of hyperoxalemia. In sum, the relative contribution of each of these unidirectional fluxes determines the overall net direction of oxalate movement across a particular segment of the intestine (Fig. 1).

The active transcellular movement of oxalate has been characterized as performed by a variety of anion exchange proteins based on their sensitivity to disulfonic stilbenes (i.e., DIDS and SITS) and the associated interactions of oxalate with the prominent anions, chloride (Cl^-), bicarbonate (HCO_3^-) and sulfate (SO_4^{2-}). In the intestine, these particular anion exchangers are responsible for Cl^-/HCO_3^- exchange, mediating Cl^- absorption and HCO_3^- secretion, as well as SO_4^{2-} absorption, and have been identified as belonging to the multi-functional SLC26 gene family where they also have an affinity for, and transport, oxalate. Of its 11 members, at least 4 SLC26 transporters are prominently expressed in the intestine and pertinent to the following discussions, they are: Slc26a1 (SAT1: Sulfate Anion Transporter 1), Slc26a2 (DTDST: DiasTrophic Dysplasia Sulfate Transporter), Slc26a3 (DRA: DownRegulated in Adenoma) and Slc26a6 (PAT1: Putative Anion Transporter 1).

The presence of simultaneous, bi-directional oxalate transport, plus the low concentrations of oxalate typically found within the body, demands a technique sensitive enough to detect and parse these opposing fluxes across the intestinal epithelium. Over the years, this has been most conveniently studied with the in vitro Ussing chamber with a range of animal models (various strains of rabbits, rats and mice) and different segments of the small and large intestine, utilizing ^{14}C-oxalate as a tracer. A variety of other experimental approaches have also been employed with these same models such as gut sacs and membrane vesicles. Other studies have included immortalized, epithelia-like human cell lines (i.e., Caco-2 and T_{84}), together with other cultured cells (e.g., HEK, Sf9 and MDCK), where transporters of interest such as the Slc26s have been either (over-) expressed or knocked-down; the *Xenopus* oocyte expression system has also been commonly used in this regard. Furthermore, the experimental conditions and how oxalate transport has been measured in all of these different systems vary too, from transepithelial fluxes and calculations of permeability, to cellular uptakes and efflux. Such diversity has produced a wealth of valuable information contributing enormously to advancing this area, but at the same time it has generated complexity and lack of consensus. As such, the data presented in the published literature necessitates careful interpretation. We recommend the reader bear this in mind when drawing their own conclusions from the following discussions.

Intestinal oxalate absorption

The favorable transepithelial electrochemical gradient that exists in vivo (i.e., typical lumen-negative potential difference and low micro-molar blood oxalate) makes the paracellular route a large contributor to absorption in this setting—depending on the amount of soluble, unbound oxalate available within the lumen and the corresponding permeability of this pathway—which will be segment specific. In general, the small intestine tends to be 'leakier' to ions and small molecules (such as oxalate) than the large intestine [22]. Under the controlled, symmetrical conditions imposed by the Ussing chamber, investigations confirmed that the absorptive flux of oxalate across the intestine, from the lumen to the blood, does indeed vary with paracellular permeability [10], but also revealed evidence of an active transcellular component that in some segments (notably, the large intestine) can result in net absorption [5, 11–13]. The relative contribution of transcellular and paracellular pathways to oxalate absorption has not been formally distinguished in vitro or in vivo [20], but a recent investigation addressing this concluded that absorption is, in fact, predominantly passive and paracellular in vitro [23]. However, this appeared just prior to another report identifying a significant contribution of DRA (Slc26a3) to transcellular oxalate absorption in the small and large intestine [17].

The discussion of Freel et al. [17] presented a number of reasons that might account for the disparities between these two studies. Among them was omission of critical electro-physiological data by Knauf et al. [23], specifically TER (TransEpithelial Resistance; Ω cm^2), or its reciprocal, conductance (G_T; mS/cm^2). G_T is a measure of the composite ionic permeability of the epithelium and the sum of transcellular conductance (G_C) and the paracellular or 'shunt' conductance (G_S). In terms of its permeability to ions, the intestine is classified as 'leaky' since G_S constitutes 53–95% of overall G_T [22]. This means that G_T is a close approximation of ionic permeability through the paracellular pathway—and thus an important indicator of epithelial integrity—so it was perplexing that this was neglected by the authors. Instead, Knauf et al. [23] utilized equivalence between oxalate permeability (P_{Ox})—calculated based on the unidirectional absorptive oxalate flux, J_{ms}^{Ox}—and the permeability of mannitol (P_{Man}), a non-metabolized solute confined to the paracellular pathway [24], as evidence of paracellular oxalate absorption.

If the assertion made by Knauf et al. [23] is indeed correct and J_{ms}^{Ox} is overwhelmingly paracellular for all regions of the intestine, then the relationship between J_{ms}^{Ox} and G_T should be direct and linear, and the intercept of this line should pass through zero. To test this, we examined the relationship between J_{ms}^{Ox} and G_T across the distal colon of wild-type (WT) mice. There was indeed a direct, linear relationship, although the predicted flux at the intercept was 9.75 ± 2.13 pmol/cm^2/h and significantly different from zero ($T = 4.58$, $P \leq 0.001$) (Fig. 2). This indicates that oxalate absorption by the distal colon is not entirely paracellular with this intercept representing 49% of the mean J_{ms}^{Ox} in Fig. 2 (19.92 ± 0.75 pmol/cm^2/h; n 85). Remarkably, this value (9.75 pmol/cm^2/h) is very similar to the proposed contribution of DRA to J_{ms}^{Ox} in this same segment (11.4 pmol/cm^2/h or 41% of J_{ms}^{Ox}) [17]. When we undertook the same analysis for DRA-knockout (KO) mice, the projected intercept was just 0.97 ± 5.91 pmol/cm^2/h, and not significantly different from zero ($T = 0.16$, P

0.870), predicting that in the absence of DRA J_{ms}^{Ox} might be paracellular. These results concur with Freel et al. [17] and earlier studies, that there is indeed an active (transcellular) component to oxalate absorption in the large intestine and this is represented by DRA. Furthermore, the comparison between WT and DRA-KO mice in Fig. 2 leads us to tentatively suggest that DRA is the sole contributor to transcellular oxalate absorption, at least in the mouse distal colon. Thus, with 41% of J_{ms}^{Ox} attributed to DRA [17], the remaining 59% of this flux would therefore be paracellular and passive.

The above analysis and the recent report by Freel et al. [17] concentrated on the lower intestine (distal ileum, cecum and distal colon), whereas the principal focus of Knauf et al. [23] has been the duodenum. It is conceivable that DRA does not participate in oxalate absorption in the proximal small intestine, even though it is prominently expressed there and responsible for 50–60% of baseline duodenal HCO_3^- secretion [25–28]. In the Ussing chamber, the duodenum, unlike more distal segments, deteriorates rapidly with observable damage to the villi after 2 h followed by their near-complete destruction at 4 h [29]. Since Knauf et al. [23] instituted a 2.5 h “equilibration period” prior to beginning their measurements, and with DRA functionally active in the villus [25, 30, 31], it is possible that they missed the contribution of DRA because the villus epithelium had largely deteriorated. This compromise to epithelial integrity would also amplify the amount of oxalate moving passively across the tissue, further diminishing their ability to detect any active, transcellular component. While 41–57% of J_{ms}^{Ox} appears to be transcellular and DRA mediated in the lower half of the mouse intestine [17], whether there is a similar contribution from DRA in the upper small intestine remains undetermined.

Intestinal oxalate secretion

Of the possible apical transporters involved in transcellular oxalate secretion, only PAT1 (Slc26a6) has been definitively identified as a participant. In the mouse small intestine, PAT1 has a prominent role in oxalate secretion by the distal ileum [16] and duodenum [18]. Previously, oxalate secretion by the rabbit ileum and colon resembled cAMP-stimulated Cl^- secretion [12, 13], involving a conductive channel in the apical membrane [32]. The centerpiece of cAMP-stimulated anion secretion by various epithelia is the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) channel [33], but when human CFTR was expressed in *Xenopus* oocytes it turned out not to transport oxalate [34]. Furthermore, unlike the rabbit, oxalate secretion by the ileum of the contemporary mouse model is not initiated by cAMP [16]. Despite this, a role for CFTR cannot be discounted; after all, cystic fibrosis patients have an elevated risk for kidney stones associated with hyperoxaluria [35] and higher than normal rates of intestinal oxalate absorption [36]. Indeed, the CFTR-KO mouse also displays hyperoxaluria, with hyperoxalemia [37]. This phenotype was recently linked to reduced expression of PAT1 in the villus duodenum and defective oxalate secretion, predicated on the ability of CFTR to facilitate Cl^-/oxalate exchange by PAT1 when the two are co-expressed in oocytes [37]. This conclusion was based on a two-third reduction in J_{sm}^{Ox} (shown as P_{Ox}) in the CFTR-KO mouse duodenum, but such an intriguing finding is unfortunately incomplete without the corresponding absorptive flux (J_{ms}^{Ox}), to determine how overall net oxalate transport was affected (Fig. 1). Using P_{Man}, Knauf et al. [37]

subtracted this from P_{Ox} to show that transepithelial oxalate secretion was all but eliminated in the CFTR-KO duodenum. However, this hinges on the critical assumption that P_{Man} is equivalent to J_{ms}^{Ox} [23] which, to the best of our understanding, has not been directly demonstrated [17], and is further complicated by a protracted 2.5 h equilibration period [37], discussed earlier. The absence of CFTR is particularly relevant in relation to DRA and its potential contribution to J_{ms}^{Ox} in the proximal small intestine. Like PAT1, DRA can also directly interact with and bind to CFTR [38–40]; plus, CFTR is required for DRA-mediated HCO_3^- secretion by the mouse duodenum [26, 41, 42]. If DRA does contribute to unidirectional oxalate absorption in the duodenum, then one cannot assume J_{ms}^{Ox} is exclusively paracellular and overlook the interaction between DRA and CFTR.

The corresponding transporter at the basolateral membrane supplying oxalate for apical secretion is unknown, but one candidate is SAT1 (Slc26a1). Primarily, an SO_4^{2-} transporter, SAT1, is the only member of the SLC26 family localized to the basolateral membrane in the intestine [19]. Recent development and study of the SAT1-KO mouse implied there was diminished intestinal oxalate secretion in the absence of SAT1, contributing to higher rates of oxalate absorption, causing hyperoxaluria, hyperoxalemia and nephrolithiasis in these animals [19]. With SAT1 present throughout the small intestine [19, 43, 44], this opened up the exciting possibility that it might be functioning in series with apical PAT1 to mediate transcellular oxalate secretion [19], a prospect explored by Ko et al. [44]. However, they were forced to concede that SAT1 was “dispensable for active oxalate secretion”, at least in the duodenum. This appeared to be confirmed after finding that the secretory oxalate flux (J_{sm}^{Ox}), expressed as P_{Ox}, was unchanged in the SAT1-KO duodenum [44]. Although proposed to be involved in oxalate secretion [19], Ko et al. [44] do not present J_{ms}^{Ox} (or any electrophysiology) and, similar to Knauf et al. [23, 37], allowed 2.5 h before commencing their experiments. As discussed earlier, such an extended period of time for the duodenum in the Ussing chamber may compromise villus integrity. Nevertheless, the hypothesis that ‘the basolateral step for oxalate secretion is mediated via exchange with SO_4^{2-} and HCO_3^- on SAT1′ [44] is intriguing. In the presence of SO_4^{2-}, we found oxalate transport by the distal ileum to be fully independent of extracellular HCO_3^- and carbonic anhydrase (CA) [45]. For the mouse, this appears to rule out a SO_4^{2-}/HCO_3^-(oxalate) exchange mechanism, at least in this segment of the small intestine. Furthermore, SAT1 mRNA is present in human Caco-2 cells [46], but the absence of SO_4^{2-} does not attenuate transepithelial oxalate fluxes [47], ruling against a prominent role for SAT1.

Even less is known about the mechanism and identities of the transporters responsible for oxalate secretion by the large intestine. In the mouse distal colon, J_{sm}^{Ox} is dependent on extracellular HCO_3^- and requires CA activity [45]. This might be suggestive of HCO_3^-/oxalate exchange at either the apical or basolateral membrane, although Whittamore et al. [45] did not probe this possibility any further with anion exchange inhibitors such as DIDS or SITS. It is uncertain how CA is involved in oxalate secretion, but the mechanism does not appear to be related to a non-catalytic role for this enzyme [45]. We considered whether the extracellular, membrane-bound isoform CA-IV might be supplying HCO_3^- for exchange with intracellular oxalate at the apical membrane. However, J_{sm}^{Ox} was unaffected when CA-

IV was targeted with a specific inhibitor, possibly implicating one of the cytosolic isoforms such as CAI or CAII [45].

The SLC26 anion exchangers and intestinal oxalate transport

While the contributions of some SLC26 anion exchangers to transcellular oxalate transport have begun to emerge, such as PAT1 and DRA, there remain many unanswered questions concerning these and, indeed, other members of this gene family expressed along the gut. There are no selective inhibitors available for the anion exchangers to help distinguish their respective roles, hence the development of KO mice and targeted knock-down (KD) of the SLC26 transporters in cultured cell models have been instrumental. The following discussion will elaborate on the intestinal SLC26 anion exchangers, considering each one in turn. For a broader perspective on the SLC26 gene family and their associated physiological roles, we direct the interested reader to a number of excellent reviews [48–51].

SAT1 (Slc26a1)

A number of studies, the majority using *Xenopus* oocytes, characterized human, rat and mouse SAT1 as a DIDS-sensitive SO_4^{2-} exchanger displaying an affinity for Cl^-, HCO_3^-, and oxalate [43, 52–56]. The report on the SAT1-KO mouse makes a compelling case for SAT1 as an important oxalate transporter, whereby oxalate uptakes into basolateral membrane vesicles made from the distal ileum, cecum and proximal colon were reduced 40–60% [19]. Furthermore, the luminal contents of the SAT1-KO cecum contained higher concentrations of SO_4^{2-} and were correspondingly lower in oxalate, consistent with the absence of a SO_4^{2-}/oxalate exchanger and impaired oxalate secretion [19]. However, as discussed above, the characteristics of oxalate fluxes by the mouse distal ileum are not compatible with a SO_4^{2-}/oxalate exchange mechanism. SAT1 is also functionally expressed at the sinusoidal membrane of hepatocytes transporting oxalate into the blood [19, 57] and at the basolateral membrane of cells in the renal proximal tubule moving oxalate from the blood into the tubular lumen [19, 52]. This expression is sex dependent, with greater functional expression in males than females [58, 59]. This appears to be under the influence of female sex hormones [58] and has been linked to the higher incidence of calcium oxalate kidney stones in men [58, 59]. However, this particular hypothesis may be undermined somewhat if the male-dominant expression of SAT1 in the liver and kidney extends to the intestine, given the evidence for its contribution to oxalate secretion in male mice [19]. With respect to the intestine, we have not encountered any differences in transepithelial oxalate fluxes between male and female mice in our collective experiments on either the distal ileum, cecum or distal colon (Whittamore, J. M. and Hatch, M., unpublished observations). Despite the disruption to oxalate homeostasis and other pathologies exhibited by SAT1-KO mice [19], this gene has not yet been associated with any human disease. However, a recent pilot study examined a small cohort of recurrent, idiopathic calcium oxalate stone formers revealing a number of sequence variants in the SAT1 gene that may be of significance [60].

DTDST (Slc26a2)

DTDST is an essential SO_4^{2-} transporter expressed prominently in chondrocytes [53, 61, 62], and mutations of this gene have been associated with a number of inherited chondrodysplasias [62–66]. There is also abundant DTDST mRNA in the small and large

intestine of mice, rats and humans [46, 53, 61, 63, 67, 68]. In human colon, DTDST is present in the upper third of the crypts, as determined by in situ hybridization and immunohistochemistry, where it is directed toward the apical membrane [61, 67]. A number of studies characterizing DTDST in oocytes have revealed its ability to transport oxalate alongside Cl^-, OH^- and possibly HCO_3^- [53, 69, 70]. These findings have therefore led to speculation on a possible role in oxalate transport, particularly the large intestine, where it might contribute to secretion by operating in either oxalate/Cl^- exchange or oxalate/SO_4^{2-} exchange modes [69, 70]. The DIDS sensitivity of DTDST appears to preclude a role in oxalate absorption, since just 50 μM was sufficient to completely inhibit mouse DTDST [70] while J_{ms}^{Ox} across the distal ileum from WT and PAT1-KO mice were unaffected by 200 μM DIDS [16].

The rate of oxalate efflux from oocytes expressing either mouse [70] or human [69] DTDST increased with extracellular pH. Raising the pH from 7.5 to 8.2 enhanced oxalate/OH^- exchange by mouse DTDST >2.5-fold [70], although recent work found J_{sm}^{Ox} by the mouse distal ileum and distal colon to be unresponsive between pH 6.9 and 7.9 [45]. Furthermore, oxalate transport by mouse, but not human DTDST [69], also displayed characteristics of inverse regulation by extracellular Cl^- [70]. Stool Cl^- concentrations within the rodent ileum and colon are low, <20 mM [71], which would likely compel oxalate absorption, given the enhanced electrochemical gradient for Cl^-/oxalate exchange, as well as reduced competition between luminal Cl^- and oxalate for uptake across the apical membrane. Indeed, reduced Cl^- concentrations are well known to affect intestinal oxalate transport [12, 13, 16, 47]. A caveat to extrapolating from oocyte studies is the reliance on the observed functional characteristics being retained when the transporter is studied as part of the native epithelium in vitro, and then subsequently in vivo. The lethality of certain DTDST mutations [65] precludes development of a KO mouse, but a ‘knock-in’ mouse possessing a non-lethal, loss-of-function mutation has been produced [66]. In humans, this particular mutation (A386V) exhibits a near 50% reduction in oxalate uptake when expressed in oocytes [69]; however, no evidence of nephrocalcinosis was seen in these transgenic mice [69].

DRA (Slc26a3)

DRA was initially considered a transporter of SO_4^{2-} and oxalate, similar to SAT-1 [72], but mutations in this gene were subsequently associated with congenital chloride diarrhea [73], a rare, inherited disease characterized by defective Cl^-/HCO_3^- exchange in the ileum and large intestine [74–76]. Until recently, the contribution of DRA to intestinal oxalate handling was unknown and development of the DRA-KO mouse [77] afforded the opportunity to examine this. Using this KO model, DRA was found to contribute significantly to transcellular oxalate absorption, accounting for 42, 57 and 41% of J_{ms}^{Ox} across the distal ileum, cecum and distal colon, respectively [17]. These reductions in oxalate absorption produced overwhelming net oxalate secretion by each of these segments, associated with a 66% decline in urinary oxalate excretion and a 60% lower plasma oxalate concentration compared to their WT counterparts [17]. Considering the limited distribution of DRA within the body, which does not extend to either the liver or kidney [68, 72, 78–82]; this further emphasizes the degree to which changes to intestinal oxalate handling can impact overall

oxalate homeostasis. We do note, however, that one study has localized DRA to the apical pole of a subset of cells in the connecting tubule and cortical collecting duct of the human nephron [83].

How this new role for DRA in oxalate absorption by the mouse intestine translates to humans is unclear. Only one study [83] has reported oxalate in patients with congenital chloride diarrhea, revealing a median urinary Ox:Cr excretion ratio well within the normal range [17]. Studies of recombinant human DRA in oocytes have indicated that it is not a convincing transporter of oxalate [84]. Although we must emphasize that oxalate transport by mouse DRA under similar experimental conditions has not been described, thus it is unclear if there are species differences in oxalate affinity, as reported for PAT1 [85]. If DRA directly mediates oxalate absorption, as indicated by the DRA-KO mouse model [17], then the transport mode will likely be Cl^-(oxalate)/HCO_3^- exchange, with oxalate directly competing with Cl^-. Consistent with this proposal, oxalate competitively inhibited $^{36}Cl^-$ uptake by rat DRA expressed in HEK cells [82]. Indeed, Cl^- strongly affects oxalate transport, this is evident in the significantly elevated J_{ms}^{Ox} across the mouse distal ileum when luminal Cl^- was removed [16], or in Caco-2 cell monolayers where mucosal Cl^- was varied between 0 and 120 mM with 50% inhibition of J_{ms}^{Ox} achieved at 20mM [47]. This competition between oxalate and Cl^- might explain why oxalate uptakes by human DRA expressed in oocytes were in fact so low where the buffers contained >100 mM Cl^- [72, 84], leading to its characterization as an unconvincing oxalate transporter.

Previously, fluxes across the PAT1-KO mouse ileum revealed a very large, disproportionate increase in J_{ms}^{Ox} that was unaffected by 200 μM DIDS [16]. In light of the contribution of DRA to J_{ms}^{Ox} [17] and its relative insensitivity to DIDS [82, 84, 86–88], this likely represented an increase in DRA-mediated oxalate absorption. This could not be explained by a transcriptional upregulation of DRA [16], but may be evidence of a direct functional coupling between DRA and PAT1 [38, 89–91]. This is not surprising given that they are both apical Cl^-/HCO_3^- exchangers with an overlapping distribution along the crypt–villus axis of the small intestine [25, 28, 30, 31, 80, 92]. In the absence of DRA, there was no corresponding increase in (PAT1-mediated) J_{sm}^{Ox} by the distal ileum [17], suggesting PAT1 does not compensate in terms of oxalate secretion in the DRA-KO ileum. This would be consistent with the lack of evidence for an upregulation of PAT1 mRNA in the small intestine of DRA-KO mice [16, 25, 27, 93, 94]. One exception is the original report on the DRA-KO mouse by Schweinfest et al. [77], who state, but do not show, there was a threefold upregulation of PAT1 in the duodenum and jejunum, and it is unclear whether they were referring to mRNA or protein.

PAT1 (Slc26a6)

There has been considerable interest in PAT1 given its demonstrated importance to intestinal oxalate secretion and consequent influence on urinary oxalate excretion [16, 18]. PAT1 is commonly thought of as being almost exclusive to the small intestine, but PAT1 mRNA is also present in the mouse and human large intestine, typically at much lower levels [38, 92, 95, 96]. In the mouse, PAT1 protein expression did not correlate with oxalate fluxes across

the large intestine [8]. To help distinguish the role of PAT1 in a human model, Freel et al. [47] examined its contribution to oxalate transport across Caco-2 cell monolayers. Utilizing an siRNA approach, PAT1 mRNA was knocked-down >75% which translated to a corresponding >60% reduction in PAT1 protein expression. This, in turn, reduced oxalate fluxes by half and, since there would have been residual PAT1, this can be considered a minimal contribution [47]. PAT1 was also shown to be a major Cl^-/HCO_3^- exchanger in this system, and its ability to mediate oxalate influx or efflux across the apical membrane was dependent on the size and direction of the prevailing Cl^- and HCO_3^- gradients [47]. Adopting another RNA interference technique, shRNA, with human T_{84} cell monolayers, Hassan et al. [97] reduced PAT1 protein expression by around 64% leading to a >57% reduction in oxalate uptake across the apical membrane, similarly showing a prominent role for PAT1 in oxalate transport in this model.

When expressed in *Xenopus* oocytes, Cl^-/oxalate exchange by human PAT1 demonstrated a far lower affinity for extracellular Cl^- than mouse PAT1 (62 mM compared to 8 mM) and mediated electroneutral (i.e., $2Cl^-/Ox^{2-}$) exchange unlike mouse PAT1 which was electrogenic (i.e., Cl^-/Ox^{2-}) [85]. These differing properties imply that the mouse may be capable of secreting oxalate into the intestine via PAT1 at low luminal Cl^- concentrations; such efficiency may explain why mice are considered “refractory” to kidney stone formation relative to humans [85]. Consistent with this, Clark et al. [85] cited that the postprandial Cl^- concentration within the human lower small intestine averaged 60 mM [98], similar to the corresponding Cl^- affinity of PAT1 (62 mM). However, this intriguing proposition is dependent on intraluminal Cl^- concentrations within the small intestine of mice being close to humans. For rats fed a standard rodent diet, the ileal contents contained ~14 mM Cl^- [71], fitting the lower Cl^- affinity of mouse PAT1 (8 mM); hence it might not necessarily be more efficient in vivo than its human counterpart.

PAT1 was not among the differentially expressed genes in the jejunum of an idiopathic hyperoxaluric rat model [99], and no human disease has yet been associated with PAT1. One common variant of PAT1 in the human population (V185M) produced a slight, but significant 25% decline in Cl^-/oxalate exchange activity in oocytes [85]. This same variant (V206M) also reduced oxalate uptake by 30% and did not cause any changes to the abundance of PAT1 in the oocyte membrane [100]. Heterozygosity for the V206M variant did not correlate significantly with plasma or urine oxalate in patients with primary hyperoxaluria or matched controls [100]. Furthermore, in a population with primary hyperparathyroidism, the homozygous V206M genotype did not associate with urinary oxalate or kidney stone prevalence [101].

Adaptations and regulation of intestinal oxalate transport

With the identification of the Slc26 anion exchangers, in particular the respective roles of PAT1 and DRA in oxalate secretion and absorption, there has been growing interest in how these transporters are regulated. The rate, mechanism and locations of electrolyte absorption and secretion along the intestine are tightly regulated and coordinated by a broad, complex multitude of local and systemic inputs integrated through the endocrine, immune and autonomic nervous systems. At the cellular level, various hormones, cytokines and

neurotransmitters can frequently exert their effects on transporter expression and activity by triggering signaling pathways often involving one or more distinct intracellular second messengers (cAMP, Ca^{2+} and cGMP) and protein kinases [102, 103]. Being able to distinguish the pathways involved in regulating oxalate transport is necessary to understand: (1) the contribution of the intestine to systemic oxalate homeostasis, (2) the pathophysiology of oxalate-associated disorders, particularly those linked to GI dysfunction, (3) how the oxalate-degrading gut bacterium *Oxalobacter* interacts with the epithelium to modify oxalate transport and (4) to assist identifying potential targets and pathways that might serve the development of future therapeutics.

Protein Kinase C (PKC) has received a great deal of attention recently, having been identified as a key negative regulator of PAT1-mediated oxalate transport, across species (human or mouse PAT1) and in different experimental systems (*Xenopus* oocytes, T_{84} cells, Caco-2 cells, native epithelium) [97, 104, 105]. Indeed, this role for PKC is not limited to mouse and human PAT1, but includes human DRA and DTDST, and the transport activity of all these exchangers can be inhibited by PKC activation in oocytes [69, 106]. There are at least 11 isoforms of PKC divided into three sub-families (conventional, novel and atypical) depending on their various requirements for activating cofactors—Ca^{2+}, diacylglycerol and phosphatidylserine [107–109]. In the gastrointestinal tract, various PKC iso-forms are involved in the development and function of the epithelial barrier, immune responses, gut motility and carcinogenesis, as well as electrolyte transport [110].

Protein Kinase C as a regulator of oxalate transport

The phorbol esters such as PMA (Phorbol 12-Myristate 13-Acetate) can mimic diacylglycerol and are widely used PKC activators [107, 111]. PMA was found to inhibit oxalate uptake by mouse PAT1 in oocytes by reducing the abundance of this exchanger at the cell membrane [104]. When PMA was subsequently applied to the isolated mouse duodenum it acutely inhibited $J_{sm}^{Ox} > 50\%$, suggesting that constitutively expressed PAT1 is regulated by a PKC-dependent pathway too [104]. In addition, PAT1 also mediates oxalate secretion by the mouse ileum [16], while DRA is involved in J_{ms}^{Ox} [17], and both of these transporters can be negatively regulated by PMA in oocytes [104, 106]. However, we found no effect of PMA on either of these unidirectional oxalate fluxes across the mouse distal ileum (Fig. 3c) and were also unable to reproduce the same inhibitory effect on J_{sm}^{Ox} by the duodenum (Fig. 3a), as previously reported [104]. Despite similarities in our respective experimental approaches, there are a number of disparities that may explain our inability to reproduce this result: (1) Hassan et al. [104] used a BALB/c mouse strain; hence, strain-related differences in responses cannot be ruled out [112]. (2) Only J_{sm}^{Ox} was presented by Hassan et al. [104]. Without the corresponding absorptive flux (J_{ms}^{Ox}), it is unclear how the net flux changes (see Fig. 1), since we found no statistically significant changes to net oxalate transport (Fig. 3a, c). (3) Hassan et al. [104] did not list calcium in their buffers for these experiments, potentially confounding their results, because the absence of extracellular calcium is well established as compromising epithelial integrity with consequences for oxalate fluxes [10]. (4) The inhibition of J_{ms}^{Ox} by the mouse duodenum was ascribed to the actions of isoform PKC-δ based on the ability of rottlerin to block the effect of PMA [104].

Not only has rottlerin been discredited as a specific PKC-δ inhibitor [113–116], but Hassan et al. [104] did not determine whether PKC-δ was expressed in their mouse model. Of the seven PKC isoforms detected in the (Swiss) mouse duodenum, PKC-δ was not among them [117]. Furthermore, this same study showed that 10 μM PMA (a concentration 50–100 times greater than used in Fig. 3) had no effect on basal duodenal HCO_3^- secretion in vitro [117], even though 20–30% of this HCO_3^- secretion has been attributed to PAT1 [26, 27, 81, 118].

Cholinergic regulation

Using the human T_{84} cell line, cholinergic regulation has been shown to be one of the specific physiological stimuli triggering PKC-δ activation, which acutely inhibits oxalate uptake in association with reduced expression of PAT1 [97], most likely through membrane endocytosis. Pre-incubation of T_{84} monolayers with the acetylcholine analog carbachol inhibited Cl^--driven oxalate uptake between 25 and 35%, a response that was attenuated by rottlerin and coincident with translocation of PKC-δ from the cell cytosol to the membrane [97]. By association, PKC-δ was concluded as responsible, but crucially Hassan et al. [97] do not show whether PKC-δ activation and the changes to PAT1 expression could actually be blocked by rotterlin after treatment with carbachol. This is important because, in T_{84} cells, carbachol has been reported to neither activate PKC-δ [119], nor are the effects of carbachol on membrane endocytosis blocked by rottlerin [120]. Nevertheless, Hassan et al. [97] projected that the direct inhibitory effects of cholinergic stimulation on T_{84} cells would translate to the native intestinal epithelium, generating excitement about the prospect of antagonizing this pathway to enhance enteric oxalate secretion [121]. However, this overlooked previous work on the autonomic control of oxalate transport where adrenergic stimulation with epinephrine reversed net secretion to absorption by the rabbit proximal colon [12]. This is consistent with the characteristic role of the enteric nervous system in regulating electrolyte transport by the mammalian intestine, whereby adrenergic agonists promote absorption and/or reduce secretion [122–125], while cholinergic stimulation conversely elicits anion secretion [126]. The actions of cholinergic agents (e.g., acetylcholine, carbachol and bethanechol) are thus more likely to promote, rather than inhibit, oxalate secretion. Unfortunately, we found that neither was the case when we tested the effects of carbachol on oxalate fluxes across the mouse distal ileum (Fig. 4a).

Purinergic regulation

In addition to being an intracellular energy source used to drive active epithelial transport, ATP and other purine nucleotides can also function as extracellular messengers regulating intestinal absorption and secretion either directly, following local cellular release, or indirectly, by acting as neurotransmitters [127, 128]. A purinergic signaling pathway has also been proposed to negatively regulate PAT1-mediated oxalate transport by Caco-2 cell monolayers via PKC-δ [105]. Luminal application of either ATP or UTP inhibited oxalate uptake by 25–30%, coincident with a 30–35% reduction in PAT1 expression at the cell membrane [105]. These responses were attenuated by the broad-spectrum PKC inhibitor Gö-6983 and targeting PKC-δ expression with siRNA [105]. However, none of the other eight isoforms (i.e., PKC-ι, PKC-βI, PKC-βII, PKC-γ, PKC-η, PKC-ζ) known to be present in Caco-2 cells [129–131] were examined, including PKC-α—associated with downregulation of the apical Na^+/H^+ exchanger NHE3 (SLC9A3) [132], and PKC-ε—

suggested to inhibit a DIDS-sensitive Cl^-/OH^- exchanger [133], in this same model. Since PAT1 mediates a large fraction of DIDS-sensitive oxalate transport in Caco-2 cells [47], we reasoned that using PMA to activate PKC should demonstrably inhibit oxalate fluxes, regardless of the isoform involved (excepting the atypical isoform PKC-ζ which is not activated by diacylglycerol [108, 110]). On the contrary, Fig. 5 shows that PKC activation does not inhibit oxalate fluxes by Caco-2 monolayers, but to our surprise elicited stimulation. Within 15 min of PMA addition, there was a sharp increase in oxalate transport which appears to peak at 60 min (Fig. 5a). A remarkably similar pattern of PKC translocation is exhibited in response to PMA in Caco-2 cells over the same time frame [134], where a rapid rise in membrane-associated PKC activity peaked at 1 min before subsequently declining, but remained elevated above baseline levels for at least 15 min (see Fig. 6a in [134]). For the remainder of the second experimental period (75–105 min), both J_{ms}^{Ox} and J_{sm}^{Ox} are, on average, 17.4 ± 2.0 and 22.3 ± 1.0 pmol/cm^2/h, and significantly higher by 24 and 18%, respectively, compared to these corresponding fluxes in the preceding control period ($P < 0.05$, Paired t test) (Fig. 5a). Accompanying these changes in oxalate fluxes is a reduction in short-circuit current (I_{sc}), indicative of net electrogenic anion secretion, and increasing paracellular permeability (G_T) which rises dramatically between 90 and 105 min (Fig. 5b). This latter observation agrees well with the reduction in TER and threefold increase in mannitol permeation previously seen by Caco-2 monolayers following exposure to 50 nM PMA [134], but contrasts with another study finding no effect of 60 nM PMA on TER [135]. The potential reasons for these disparities were discussed by Turner et al. [135] and they are clarified no further by our data, but a number of PKC isoforms (α, βI, and η), which are associated with the regulation of Caco-2 cell permeability [110, 131], will have been activated by PMA. Whether the increases to oxalate transport in Fig. 5a represent movement through the paracellular pathway cannot be determined. However, we note that the stimulation of oxalate fluxes between 60 and 105 min does not synchronize with G_T as one might expect if this was entirely due to changes in paracellular oxalate movements.

Reciprocal regulation of oxalate and citrate transport

An intriguing interaction has been revealed between oxalate transport by PAT1 and citrate transport by the sodium dicarboxylate cotransporter, NaDC1 (SLC13A2) [136]. Urinary citrate is an important chelator of calcium, consequently reducing the potential for calcium and oxalate to interact. Citrate thus exerts a protective effect by limiting calcium oxalate crystal formation and growth; hence hypocitraturia, like hyperoxaluria, is a major risk factor for kidney stones [137, 138]. Based on this relationship, Ohana et al. [136] hypothesized that oxalate and citrate levels are coordinated by their respective transporters, namely PAT1 and NaDC1, which are localized to the apical membrane in both the proximal tubule and small intestine [92, 139, 140]. This was supported by the observations that PAT1-KO mice, in addition to being hyperoxaluric, also display hypocitraturia [136, 141]. The reduction in urinary citrate in these animals corresponded to increased Na^+-dependent succinate uptake by kidney cortical slices (70%) and isolated sheets of jejunum (35%), considered representative of increased NaDC1 activity, implying that the absence of PAT1 enhanced renal citrate recovery and intestinal citrate absorption [136]. Suspecting there might be a functional interaction between NaDC1 and PAT1, Ohana et al. [136] went on to show that co-expression of mouse PAT1 with either opossum or human NaDC1 in oocytes enhanced

PAT1-mediated oxalate transport, while Na^+-dependent succinate transport by NaDC1 was concomitantly reduced [136]. This reciprocity was determined to be the result of a direct physical and functional interaction between these transporters and suggested to serve as a mechanism for sensing and regulating urinary oxalate and citrate levels [136], which may be relevant to the changes in renal citrate and oxalate handling seen during a metabolic acidosis [142]. It was notable that DRA, but not DTDST, also inhibited succinate uptake by NaDC1 in a similar manner to PAT1 in oocytes [136]. The significance that such reciprocal regulation between PAT1 (or indeed DRA) and NaDC-1 might have for oxalate transport by the small intestine was not considered.

Adaptations to acid–base status

Even though the mammalian intestine does not directly participate in maintaining acid–base balance, it makes a major, clinically relevant contribution [143, 144] and also responds to acid–base abnormalities, most prominently through modifications to sodium chloride (NaCl) cotransport [145, 146]. These consist of acute, reversible responses by the apical Na^+/H^+ and Cl^-/HCO_3^- exchangers to variations in systemic pH, HCO_3^- and CO_2, in conjunction with CA, in a distinct, segment-specific manner [145–148]. Since DRA and PAT1 are the main Cl^-/HCO_3^- exchangers involved in intestinal oxalate transport [16–18], this raised the question of whether oxalate also displays characteristics of regulation by acid–base variables, particularly since PAT1-mediated HCO_3^- secretion appears sensitive to short-term (<1 h) systemic acid–base disturbances [41]. We recently explored how oxalate transport by the mouse distal ileum and distal colon responded to acute alterations in buffer pH, HCO_3^- and CO_2 in vitro, and the involvement of CA [45]. Contrary to expectation, net oxalate secretion by the distal ileum, which involves PAT1 [16], was completely insensitive to any changes in pH, HCO_3^- or CO_2, and unaffected by CA inhibition. This latter observation is noteworthy given that PKC disrupts the physical interaction between CAII and PAT1 in HEK cells [149]. Clearly, no such arrangement is needed to support oxalate secretion in the mouse ileum. In contrast, J_{sm}^{Ox} by the distal colon required CA activity, was dependent on the presence of extracellular HCO_3^-/CO_2 and could be acutely stimulated by increasing PCO_2, but was impervious to pH [45], suggesting colonic oxalate transport may indeed be responsive to systemic acid–base disorders. This was confirmed by a report of net oxalate absorption being completely reversed to net secretion by the rat distal colon following induction of a chronic metabolic acidosis [142]. The initiation of colonic oxalate secretion was associated with a dramatic 75% reduction in urinary oxalate excretion [142]. Importantly, we do not discount the impact of an acidosis on renal oxalate handling, especially since clearance ratios indicated enhanced net re-absorption by the kidneys, yet plasma oxalate levels remained stable [142]. Unless hepatic oxalate production was diminished by this protracted acidosis, as suggested by Bushinsky et al. [150], these circumstances necessitate an alternate route for elimination, coinciding with the initiation of colonic oxalate secretion [142]. These adaptations during a metabolic acidosis offer additional evidence for a dynamic gut–kidney axis in the management of oxalate excretion alongside chronic renal failure [3, 5, 6], oxalate loading [151] and colonization with *Oxalobacter* [7–9]. Even though the signaling pathways and transporters involved remain unidentified, these new findings offer a glimpse at the presence of a previously unrecognized regulatory mechanism. How this might translate to humans is presently unclear. An acute

acid load reportedly increased urinary oxalate and the risk for calcium oxalate crystallization [152], while the impacts of drugs which antagonize CA activity (potentially generating an acidosis) are limited and inconclusive. For example, a significant rise in urinary oxalate was apparent for patients taking acetazolamide, but this did not exceed the normal range [153], while studies of other CA-inhibiting drugs revealed no effects on urinary oxalate excretion [154–156].

Hyperoxaluria following gastric bypass

With the increasing prevalence of obesity and the modern bariatric surgical procedures implemented in an effort to reduce body weight, urinary tract stone disease is in the top ten primary diagnoses for post-operative conditions [157]. A number of clinical reports have suggested the hyperoxaluria associated with Roux-en-Y gastric bypass (RYGB) could be explained by enteric hyperoxaluria caused by steatorrhea [158]. The mechanistic basis of RYGB-induced hyperoxaluria was directly addressed using an RYGB/SHAM obese rat model [159]. The results show that oxalate bioavailability (for intestinal absorption) is largely dependent on the fecal fat content and there is a threshold level for fecal fat which, when reached, significantly alters colonic permeability to oxalate. Clearly, there is an effect of steatorrhea on both the paracellular and transcellular movement of oxalate in the distal colon and each pathway may involve different signaling mechanisms which remain unexplained at present. It was concluded that passive, paracellular oxalate absorption and more importantly oxalate bioavailability are promoted by steatorrhea following RYGB. A novel finding was that the Roux limb is not hyper-absorbing oxalate, which was previously unknown. In fact, both unidirectional and net oxalate fluxes are abolished in the Roux limb compared to robust oxalate fluxes across that segment of intestine when left intact. This study underscores the need for examining other intestinal segments, surgically reconfigured or not, for their contributions to enteric hyperoxaluria following bariatric procedures.

Gut microbiota and oxalate homeostasis

A number of intestinal bacteria degrade oxalate and these are divided into two groups: (1) the “generalist oxalotrophs”, including some strains of *Bifidobacterium* and *Lactobacillus*, which can utilize oxalate in addition to other carbon sources and (2) the “specialist oxalotrophs”, such as *Oxalobacter formigenes*, a commensal anaerobe that relies on oxalate as its sole carbon source [15, 160]. This section primarily focuses on *Oxalobacter* in laboratory rodents since it has been the most intensely studied with respect to mammalian oxalate homeostasis and intestinal transport. For background information, we recommend the section on the role of oxalate-degrading bacteria from a previous review [21] for the following discussion, in addition to other recent reviews bearing relevant sections on this topic [161–164].

Oxalate-degrading bacteria in humans

Human studies [165–168] and earlier work [21, 163] have focused primarily on *Oxalobacter*, in addition to some commercial probiotic preparations of various formulations of *Lactobacillus* and *Bifidobacterium* strains (Oxadrop, VSL#3, and including a symbiotic preparation Agri-King Synbiotic [167]). However, these studies have shown inconsistent

results. Potential reasons for the conflicting and sometimes transient probiotic effects may include a lack of standardization of the various formulae and poor control of dietary oxalate and calcium. Indeed, in vitro analysis of one commercial probiotic showed it contained no viable oxalate-degrading bacteria [169]. More rigorously designed clinical studies including patient groups with various etiologies of hyperoxaluria are warranted to reconcile the beneficial probiotic effects in reducing hyper-oxaluria and hyperoxalemia evident in laboratory animals with the inconsistent probiotic effects observed in human studies. In addition, the importance of determining how colonization with beneficial oxalate degraders is established and maintained, as well as the intraluminal factors affecting colonization (apart from the obvious antibiotic treatments), is essential for a therapeutic approach utilizing probiotics to be realized. Finally, it cannot be overstated that methodologies for the collection and measurements of oxalate in blood and urine are in need of rigorous attention and quality control across all studies, animal and human.

Oxalobacter formigenes in laboratory animals

Initial studies in rats using a variety of experimental approaches showed that *Oxalobacter* can derive oxalate from systemic sources by inducing active intestinal oxalate secretion [7], followed by intraluminal enzymatic oxalate degradation. The latter is important to provide a concentration gradient for the movement of oxalate into the gut lumen, including through the passive paracellular pathway (Fig. 1), which may be especially enhanced in cases of hyperoxalemia. The beneficial consequences of these combined actions of *Oxalobacter* will result in an efficient enteric secretion leading to a reduced renal oxalate burden [7–9]. These actions of *Oxalobacter* were also recently confirmed in an obese rat model following bariatric surgery resulting in a >70% reduction in urinary oxalate excretion over an 8-week period following a combined manipulation in dietary fat and colonization with the wild rat strain, OxWR [158]. The beneficial probiotic effect is perhaps best highlighted in a KO mouse model of primary hyperoxaluria, type 1, having a deficiency of the liver enzyme Alanine–Glyoxylate-aminoTransferase (AGT). Artificially colonizing AGT-KO mice resulted in a normalization of both blood and urinary oxalate levels in otherwise hyperoxalemic/hyperoxaluric animals. The basic mechanism of action of *Oxalobacter* (both OxWR and the human strain, HC-1) was confirmed to be alterations in the net directional movements of oxalate resulting in enteric oxalate elimination [9]. Although attempts were made to identify the transport proteins involved, this proved difficult due to the lack of specific primary antibodies to the SLC26s. Currently, these questions are being addressed using single KO mouse models of SAT1, DRA and PAT1.

More in-depth studies with mice have revealed *Oxalobacter* can colonize various segments of both the large and small intestine for varying time periods and are more persistent in the colon compared to more proximal segments [9]. It is notable that urinary oxalate excretion remains persistently low over a 75-day colonization period in WT mice, presumably due to development of a highly robust colonization and possibly adaptations in the efficiency of oxalate transport [8]. In addition, modulation of oxalate secretion by *Oxalobacter* was also evident in the distal ileum and cecum, but curiously absent in the proximal colon, of both rats and mice [7, 9]. The refractory nature of this latter segment has not been explained and warrants further study given so little is known about the bacterium–host interaction. About a

decade ago, we proposed that the mechanism of action for *Oxalobacter* was possibly due to elaboration of a bacterial secretagogue, since delivery of an encapsulated and freeze-dried, cell-free *Oxalobacter* lysate had the same effect on colonic oxalate transport as found in animals colonized with *Oxalobacter* [7]. However, reproducing this effect in vitro has not been successful in our hands despite testing multiple preparations including whole bacterial cells, cell membranes and heat-treated lysates [7]. A confounding issue is the oxalate-degrading activity of *Oxalobacter* enzymes and degradation of the ^{14}C-oxalate tracer used to measure intestinal fluxes together with the highly oxygenated buffers used in Ussing chamber experiments [7]. A number of intriguing recent reports have shown that exposure to *Oxalobacter* conditioned media (CM) at a 1:50 dilution for 6–24 h stimulates oxalate influx by Caco-2 cells and a $^{>}$4-fold increase in DRA mRNA expression, with no changes to DTDST or PAT1 [170, 171]. We repeated this experiment measuring unidirectional oxalate fluxes across Caco-2 monolayers in Ussing chambers using a 1:50 dilution (and also a 1:25 dilution; see Supplementary Table 1) of CM from the cultured human strain of *Oxalobacter*, HC-1. Exposing the mucosal compartment of these monolayers to CM from *Oxalobacter* for 18–24 h had no effects on oxalate transport (Fig. 6). In addition to these contrasting results, it is difficult to reconcile why *Oxalobacter* CM targets DRA [170, 171], and not PAT1, since the latter is the major oxalate transporter in Caco-2 cells [47], and given the respective contributions of PAT1 and DRA to oxalate secretion and absorption in mice [16–18]. It is also notable that Hassan et al. reported no effect of CM from *Lactobacillus acidophilus* [170, 171], whereas several studies have previously shown that *Lactobacillus* significantly upregulates the functional expression of DRA in both Caco-2 and mouse models [172–174].

Bifidobacterium and oxalate homeostasis

Recently, we studied the efficacy of the probiotic *Bifidobacteria* sp. and their contribution to oxalate homeostasis in WT and AGT-KO mice. Groups of mice were artificially colonized with one of two *Bifidobacteria* strains, an oxalate-degrader *B. animalis* and a non-degrader *B. adolescentis* [175]. As anticipated, urinary oxalate was reduced in both WT (↓44%) and AGT-KO (↓33%) mice receiving *B. animalis*. However, the question was whether *B. animalis* achieved this by altering intestinal oxalate transport, which is the major characteristic of *Oxalobacter*. Neither of the two *Bifidobacteria* strains had significant effects on net oxalate transport in the three segments examined (distal ileum, cecum and distal colon). Thus, the reductions in urinary oxalate in animals colonized with *B. animalis* occurred solely by intraluminal oxalate degradation. It is notable here that Kumar et al. [176] looked at three other strains of *Bifidobacteria* and reported an increased expression of DRA in both mice and Caco-2 cells. This work focused on Cl^- uptake by Caco-2 monolayers using a surrogate tracer (^{125}I) to show an approximate twofold induction of DRA function, although we saw no clear changes to the DRA-mediated absorptive oxalate flux, J_{ms}^{Ox} [175]. The current work in our laboratory is addressing the same question of *Lactobacillus* sp., since the ability to induce net intestinal oxalate secretion by interacting with the host enterocyte appears to be a trait unique to *Oxalobacter*. A prior study by Murphy et al. [177] has already shown that *Lactobacillus animalis* significantly reduces urinary oxalate excretion in rats, but the effects on intestinal transport of oxalate were not the focus of this work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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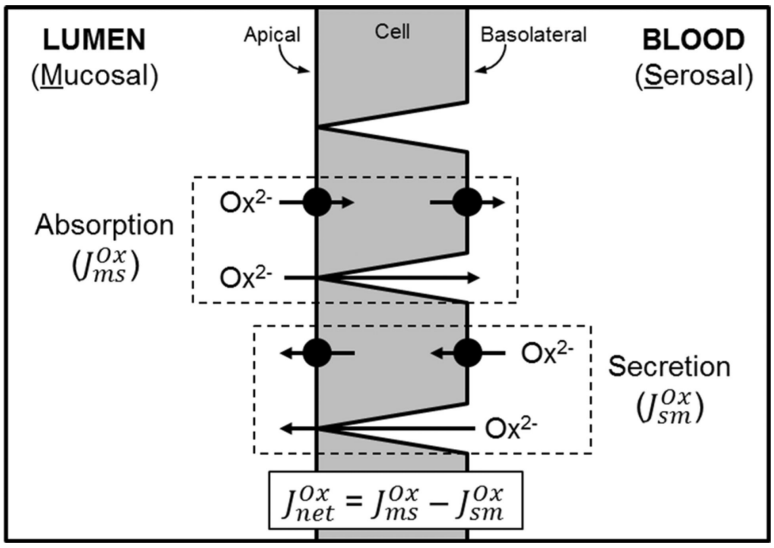

Fig. 1.

Pathways for oxalate (Ox^{2-}) transport across the intestinal epithelium. An illustration of the unidirectional absorptive (J_{ms}^{Ox}) and secretory (J_{sm}^{Ox}) oxalate fluxes, which are composed of both transcellular and paracellular pathways. Active, transcellular oxalate movements are facilitated by transport proteins, such as the SLC26 anion exchangers, expressed in the apical and basolateral membranes. The paracellular component involves oxalate moving passively through the tight junctions and between cells in response to the prevailing electrical and concentration gradients across the epithelium, and relative ionic permeability of this pathway. These processes of absorption and secretion occur simultaneously across the epithelium; and in the Ussing chamber they can be directly measured and used to calculate the overall net direction of oxalate movement (J_{net}^{Ox}) across the epithelium. See text for further details

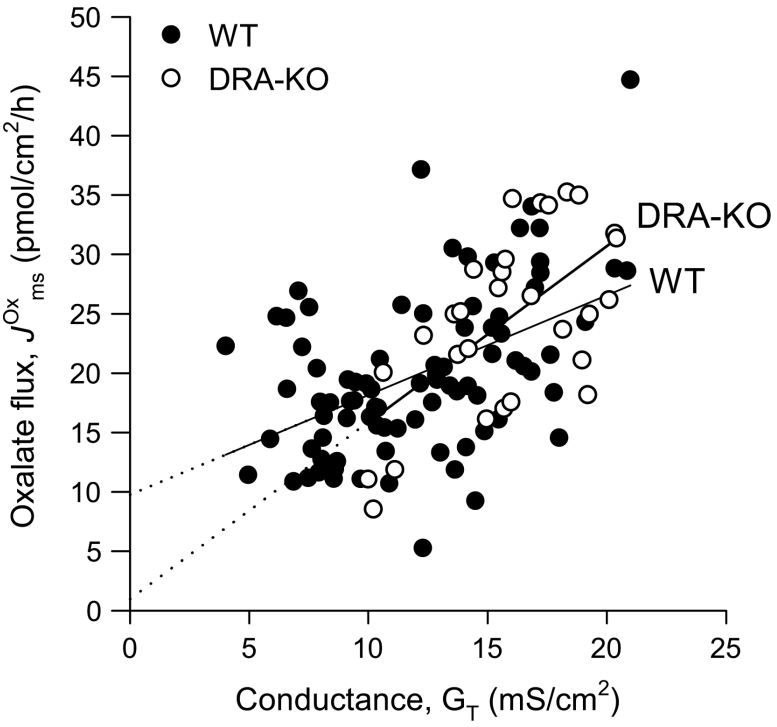

Fig. 2.

The relationship between transepithelial conductance, G_T and the unidirectional absorptive oxalate flux, J^{Ox}_{ms}, across the distal colon from WT (*filled circle*) and DRA-KO (*open circle*) mice. Individual data points were collated from published studies [17, 45] together with the results from previously unpublished studies in our laboratory. J^{Ox}_{ms} and G_T were measured on isolated segments of the distal colon mounted in the Ussing chamber under symmetrical, short-circuited conditions with standard bicarbonate buffer. Further methodological details are available in the supplementary material. The accompanying linear regression equation and analysis summary for WT mice is: $J^{Ox}_{ms} = 9.75 + (0.84 \times G_T)$ ($R^2 = 0.233$) and $F_{1,83} = 25.15$, $P < 0.001$. The regression equation and analysis summary for DRA-KO mice is: $J^{Ox}_{ms} = 0.97 + (1.49 \times G_T)$ ($R^2 = 0.378$) and $F_{1,27} = 16.40$, $P < 0.001$

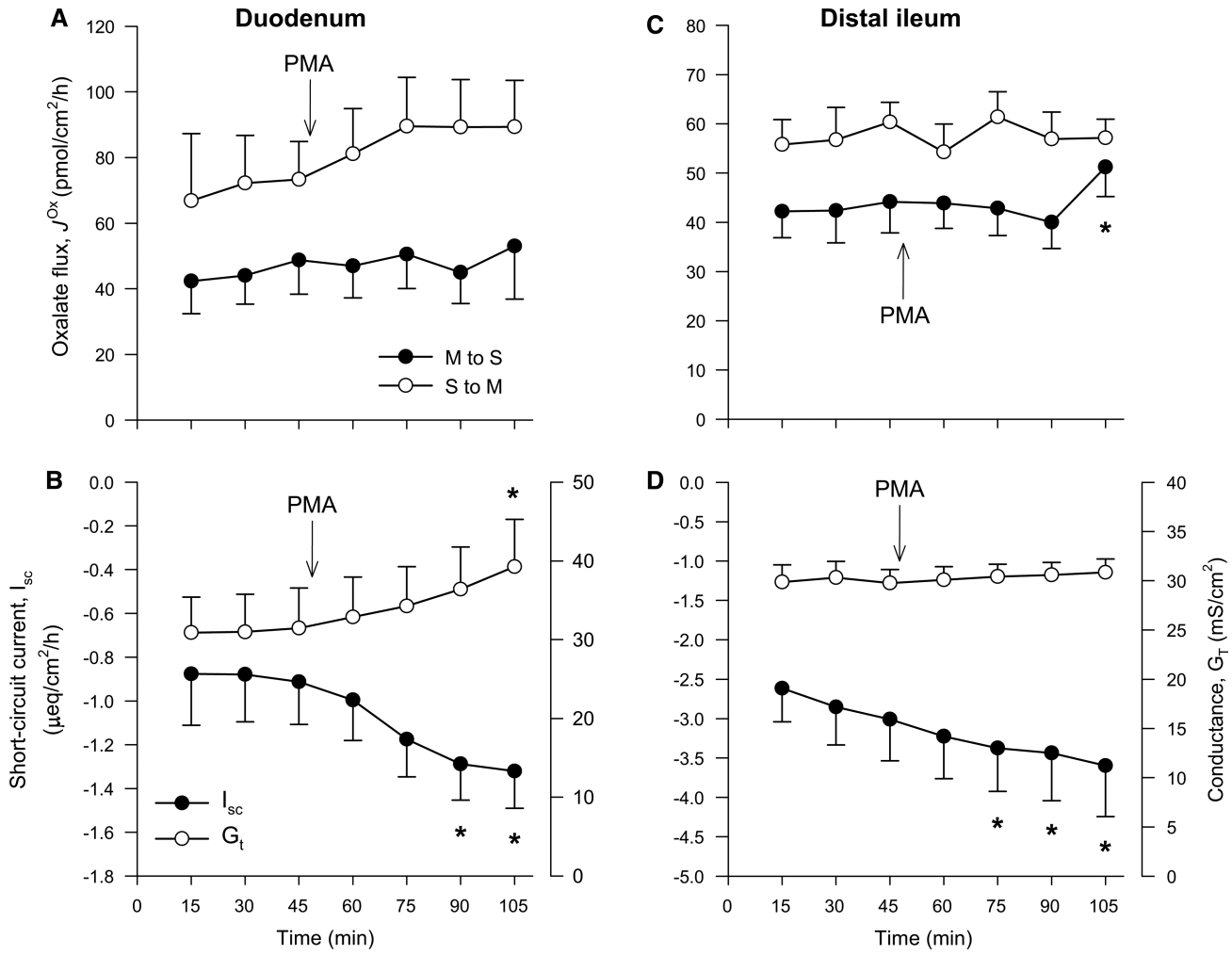

Fig. 3.

The Protein Kinase C (PKC) activator phorbol-12-myristate-13-acetate (PMA) does not inhibit oxalate transport by the mouse small intestine in vitro. The unidirectional oxalate fluxes measured across isolated short-circuited segments of the duodenum (**a**) and distal ileum (**c**) following the addition of PMA (100 or 200 nM) to the mucosal and serosal baths under symmetrical buffer conditions. The accompanying panels show the associated measures of short-circuit current, I_{sc} (*filled circle*) and transepithelial conductance, G_T (*open circle*) for duodenum (**b**) and distal ileum (**d**). Each data point represents the mean ± SE from $n = 6$ (duodenum) and $n = 8$ (distal ileum) G_T-matched tissue pairs. An *asterisk* indicates a statistically significant difference from the preceding control period (0–45 min) determined by repeated measures one-way ANOVA followed by multiple, pairwise post hoc comparisons. Further details are available in the supplementary material. There were no differences between any of the transport characteristics in the presence of either 100 or 200 nM PMA; hence, these data sets were combined to produce this figure

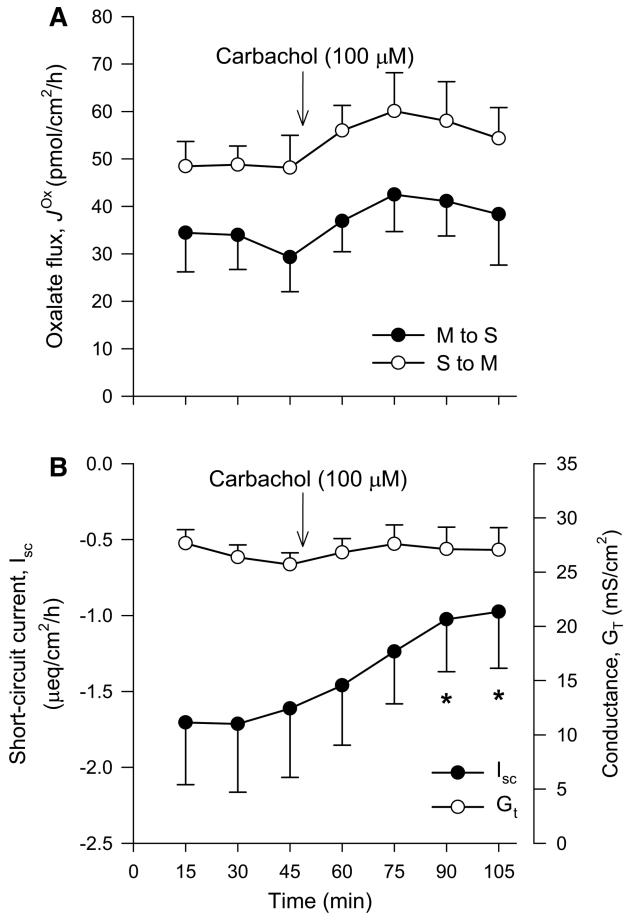

Fig. 4.

Cholinergic stimulation does not inhibit oxalate transport by the mouse distal ileum in vitro. The unidirectional oxalate fluxes measured across isolated short-circuited segments of the distal ileum (**a**) following the addition of carbachol (100 μM) to the serosal bath under symmetrical buffer conditions. The accompanying measures of short-circuit current, I_{sc} (*filled circle*), and transepithelial conductance, G_T (*open circle*), are shown in **b**. Each data point represents the mean ± SE from $n = 6$ G_T-matched tissue pairs. An *asterisk* indicates a statistically significant difference from the preceding control period (0–45 min) determined by repeated measures one-way ANOVA followed by multiple, pairwise post hoc comparisons. Further details are available in the supplementary material

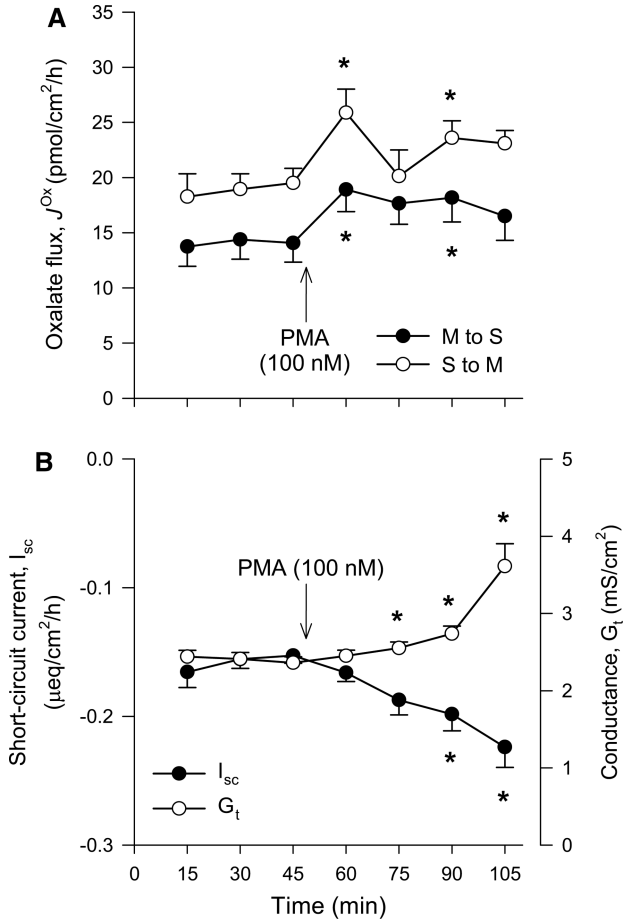

Fig. 5.

The Protein Kinase C (PKC) activator phorbol-12-myristate-13-acetate (PMA) stimulates oxalate transport by human Caco-2 cell monolayers. **a** displays the unidirectional oxalate fluxes measured across cultured Caco-2 cell monolayers at 19–24 days post-seeding under symmetrical, short-circuited conditions following the addition of PMA (100 nM) to the mucosal and serosal baths. The accompanying measures of short-circuit current, I_{sc} (*filled circle*), and transepithelial conductance, G_T (*open circle*), are shown in **b**. Each data point represents the mean ± SE from $n = 9$ G_T-matched pairs of monolayers. An *asterisk* indicates a statistically significant difference from the preceding control period (0–45 min) determined by repeated measures one-way ANOVA followed by multiple, pairwise post hoc comparisons. Further details are available in the supplementary material

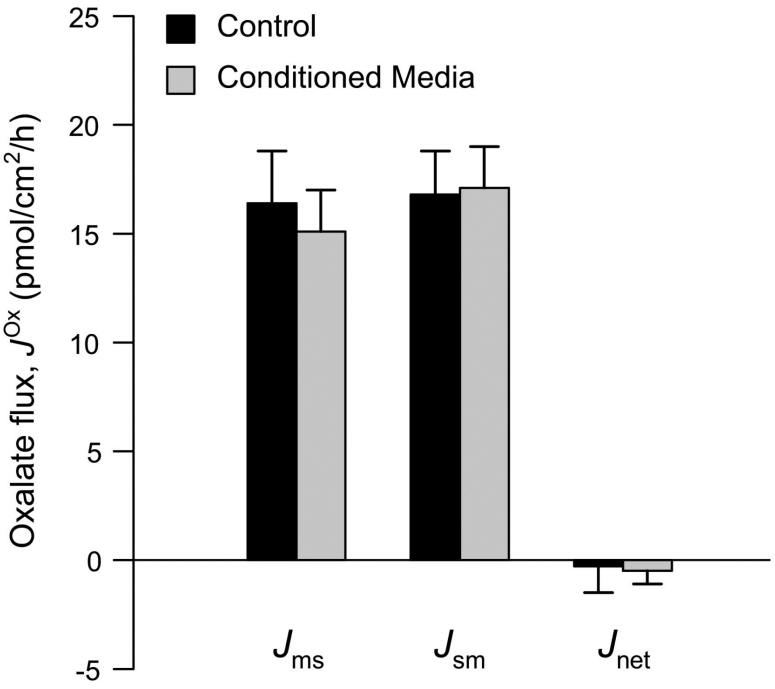

Fig. 6.

Exposing human Caco-2 cell monolayers to 'Conditioned Media' (CM) from a culture of *Oxalobacter formigenes* does not significantly enhance oxalate transport. The unidirectional oxalate fluxes measured across cultured Caco-2 cell monolayers at either 11 or 28 days post-seeding under symmetrical, short-circuited conditions following prior mucosal (luminal) exposure to 'conditioned media' from an O/N culture of *Oxalobacter* strain HC-1 (1:50 dilution) for 18–24 h are shown alongside concurrent controls exposed to sterile media only. There were also no significant differences to the electrophysiological parameters. Short-circuit current (I_{sc}) was −0.09 ± 0.02 μeq/cm^2/h (control) compared to −0.09 ± 0.02 μeq/cm^2/h (conditioned media), and transepithelial conductance (G_T) was 6.2 ± 0.4 mS/cm^2 (control) compared to 6.4 ± 0.3 mS/cm^2 (conditioned media). The data represent the mean SE from a total of n = 16 and n = 20 G_T-matched pairs of monolayers for the control ± and conditioned media treatments, respectively. Further details are available in the supplementary material. There were no statistically significant differences between any of the transport characteristics of monolayers at 11 days compared to 28 days post-seeding; hence, monolayers from these two time points were combined to produce this figure