Renal and Intestinal Handling of Oxalate following Oxalate Loading in Rats

Marguerite Hatch Robert W. Freel
Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, Fla., USA

Key Words
Hyperoxaluria · Hyperoxalemia · Renal failure · Angiotensin II · Losartan · Distal colon · Chloride · Transport

Abstract
Background: The enteric excretion of oxalate has been established in rats with chronic renal failure induced by 5/6 nephrectomy [Hatch et al.: Regulatory aspects of oxalate secretion in enteric oxalate elimination. JASN 1999;10:S324] and this response is mediated by angiotensin II receptor activation. However, the renal and intestinal handling of oxalate has not been evaluated for other common models of hyperoxaluria that simulate primary hyperoxaluria or oxalate stone disease. Methods: We assessed the renal clearances of creatinine, oxalate and calcium in three rat models: chronic hyperoxaluria (CH), chronic hyperoxaluria with hyperoxalemia (CHH) and acute hyperoxaluria (AH), and evaluated the transepithelial transport of oxalate and chloride in large intestinal segments of these models and their sensitivity to angiotensin II antagonism. Results: Hyperoxaluria alone (CH) was not associated with changes in colonic oxalate transport, whereas changes in net oxalate transport in distal colon from absorption to net secretion was observed in models with hyperoxalemia (CHH and AH). Angiotensin II receptor antagonism with losartan reduced net colonic oxalate secretion in AH but not CHH. Conclusions: Colonic secretion of oxalate is stimulated in rat models exhibiting hyperoxalemia suggesting a contribution of this extrarenal pathway to regulation of oxalate mass balance in clinical conditions manifesting hyperoxalemia. The transport avenues and regulatory mechanisms may not be identical to those observed during adaptive enteric oxalate secretion in chronic renal failure models.

Introduction
The healthy kidney is the primary route for the excretion of oxalate, whether it is derived from dietary absorption or endogenous metabolism [1]. There is, however, a small amount of oxalate normally excreted by an enteric route which can be significantly enhanced when renal function is compromised [2, 3]. Whereas the normal rat distal colon consistently supports a net absorptive flux of oxalate, this is reversed to net secretion in rats with chronic renal failure (CRF) induced by either 5/6 nephrectomy [3] or by chronic hyperoxaluria [4]. In addition, our previous studies implicated the involvement of angiotensin II (ANG II) in mediating this adaptive response in both CRF models [4, 5]. The notion that enteric handling of oxalate can participate in the mass balance of
oxalate is further suggested by the fact that the magnitude and direction of oxalate transport across intestinal epithelia can be regulated [6–8]. Thus, it appears that oxalate excretion is balanced between the renal and enteric routes depending upon the degree of renal sufficiency. It is less clear how these two excretory routes are coordinated, if at all, in the context of various oxalate-associated diseases such as in primary hyperoxaluria or oxalate stone formation. In this report we present results of experiments designed to examine both colonic and renal handling of oxalate in several rat models that simulate some of the clinical characteristics of the oxalate-associated diseases.

Materials and Methods

Animal Models

Male Sprague-Dawley rats (~300 g) were used in these studies. Since hyperoxaluria and oxalate stones can be initiated by feeding oxalate salts in the food, administering ethylene glycol in the drinking water, or by an intraperitoneal injection of oxalate [9], we divided rats into these three treatment groups. Untreated rats served as controls. The rats had free access to drinking water that was untreated or treated with ethylene glycol and with unlimited access to Purina rat chow 5001. The chronic hyperoxaluric (CH) model had a specialized diet to include oxalate (see below). At the end of the specified treatment, prior to collecting blood and colonic tissues for the flux studies, urine from all rats was collected into vessels containing 3 ml of 3.5% HCl over a period of 24 h. In the acute hyperoxaluric (AH) group, urine was collected directly from the bladder, using a syringe, at the time the rats were killed for the flux studies. At the end of each treatment regimen, the animals were euthanized by an intraperitoneal injection of sodium pentobarbital (150 mg/kg) and promptly exsanguinated by cardiac puncture. The blood was handled immediately with the appropriate precautions to prevent oxalogenesis [10]. Oxalate was measured in plasma and urine using an enzymatic assay procedure routine in our laboratory [10, 11]. Creatinine and calcium were determined in the urine and plasma samples using the Sigma kit assay 555A and Sigma kit assay 587-A, respectively (Sigma Chemical Co., St. Louis, Mo., USA).

The rat models were developed as follows: (1) Chronic hyperoxaluria (CH) without hyperoxalemia was produced in rats by feeding oxalate in a low Ca2+ diet for a 3-week period. A powdered diet containing 0.01% Ca2+ (Product No. TD 99354, Harlan-Teklad, Madison, Wisc., USA) was supplemented with ammonium oxalate (0.5%) and thoroughly mixed into batches before lightly wetting the mixture and rolling it into balls. The food balls were dried at room temperature and then placed in the rat cages. (2) Chronic hyperoxaluria and hyperoxalemia (CHH) were induced in rats by adding 0.75% ethylene glycol to their drinking water for a period of 4 weeks. This regimen has been documented to produce persistent calcium oxalate crystalluria by day 7 and nephrolithiasis by 2–3 weeks [9]. (3) Acute hyperoxaluria (AH) was induced in rats by administering an intraperitoneal injection of sodium oxalate (3 mg/100 g body weight). Tissues were removed from this group 6 h after the injection for the flux studies since Khan [9] reported that urinary oxalate excretion is maximal at this time.

Flux Studies

Immediately following euthanasia and exsanguination of the rats, the proximal and distal colonic segments were removed, cleansed in ice-cold saline and partially stripped of the serosal muscularis. Flat sheets of tissue were mounted in modified Using chambers with an exposed tissue area of 0.64 cm2. Transepithelial fluxes of oxalate and chloride were measured using 14C-oxalate and 36Cl across colonic tissues bathed on both sides by 10 ml of buffered saline (pH 7.4) at 37°C circulated by bubbling with 95% O2/5% CO2. The standard saline contained the following solutes (mmol/l): 139.4 Na+, 5.4 K+, 1.2 Mg2+, 123.2 Cl−, 21.0 HCO3−, 1.2 Ca2+, 0.6 H2PO4−, 2.4 HPO42−, and 10 glucose. The magnitude and direction of the net flux (Jnet) was determined by calculating the difference between two unidirectional fluxes (mucosal to serosal, Jmuc and serosal to mucosal, Jmuc) measured for a control period of 45 min (Per I) at 15-min intervals, under short-circuit conditions. Per I was followed by a second 45-min flux period (Per II) in order to determine either time-dependent effects, or effects of drug (losartan, at 10−3 M) addition. The electrical parameters of the tissue were also recorded at 15-min intervals throughout the entire experiment. Tissue conductance (GT, mS cm−2) was calculated as the ratio of the open-circuit potential (mV) to the short-circuit current (Is, μA cm−2). 14C-oxalate and 36Cl were obtained from New England Nuclear (Boston, Mass., USA). The AT1 receptor antagonist, losartan, was a gift from Merck & Co., Inc. (Rahway, N.J., USA), and all other reagents were purchased from Sigma Chemical Co.

Statistical Analysis

Statistical analysis of the data derived from these experiments was performed by using a one-way analysis of variance (ANOVA) followed by Bonferroni’s t test for multiple comparisons with the control group. A paired or unpaired t test was used for the comparison of two means. Differences were considered significant if p ≤ 0.05.

Results

Plasma Solute Concentrations and Renal Excretion

The results presented in figure 1 provide a comparison of plasma solute concentrations and urinary solute excretion in all groups. Creatinine measurements were used to provide an index of renal function in these different animal groups and calcium measurements were included to evaluate the effects of oxalate loading on this important stone-risk factor. Renal handling of oxalate is characterized here using plasma and urinary measurements and standard calculations for renal solute clearances. As mentioned in the Methods section, 24-hour urinary solute excretion and renal clearances were not determined in the AH group, but these parameters are presented for the other groups and controls.

It is apparent that plasma creatinine, urinary creatinine excretion and clearance in both CH and CHH rats were not significantly different from control. These results suggest that renal function was unaffected by the

Renal and Intestinal Oxalate Excretion in Rats

Am J Nephrol 2003;23:18–26
oxalate loads delivered to these two groups. In contrast, a small but significant increase in plasma creatinine occurred in the AH group indicating some degree of renal dysfunction.

Oxalate Handling

Of the three oxalate-loaded groups examined, plasma oxalate concentration was found normal in CH rats, but significantly elevated in both CHH and AH compared to control. As expected, urinary oxalate excretion was markedly elevated in all of the treated groups. In the AH group, the magnitude of hyperoxaluria was estimated by measuring oxalate (µmol/l) in the variable, small volumes of urine retrieved from each rat bladder at the time of euthanasia. Urinary oxalate was standardized per unit of creatinine (µmol/l) that was also measured in the same bladder urine specimen. A group of rats injected with isotonic saline served as controls. This excretion ratio (oxalate/creatinine) determined in the AH rats (n = 11) increased about 9-fold to 230 ± 130 from a value of 26 ± 2 which was determined from the paired controls (n = 9) injected with saline. The results also show that the renal clearance of oxalate was markedly increased in CH by 28-fold compared to control. However, despite a 7-fold increase in renal clearance of oxalate in CHH, this did not reach statistical significance. Finally, clearance ratios (oxalate/creatinine) that are significantly greater than unity in CH (10.2 ± 1.2, n = 7) and CHH (2.1 ± 0.5, n = 16) rats indicate varying degrees of tubular oxalate secretion in both hyperoxaluric groups compared to apparent tubular reabsorption in the controls (0.5 ± 0.1, n = 18).
Calcium Handling

No differences in plasma calcium concentrations were observed in any of the treated groups compared to control, however, renal handling of calcium was altered to varying degrees. A significant reduction in urinary calcium excretion was evident in the CH group which was most likely due to the low calcium content of the oxalate-supplemented diet. In AH rats, urinary calcium excretion was also reduced by about 50%. This decrease was similarly determined in bladder urine removed from the AH group as described above for oxalate. (Urinary calcium was standardized per unit of creatinine (μmol/l) measured in the same bladder urine specimen from AH and rats injected with isotonic saline.) The mean urinary excretion ratio (calcium/creatinine) determined in the AH rats (n = 11) decreased to 0.22 ± 0.04 from a value of 0.43 ± 0.06 determined in the paired controls (n = 9). Urinary calcium excretion in CHH rats was not significantly different compared to controls. It is also apparent that renal clearance of calcium is markedly reduced in CH rats. A calculation of renal clearance ratios (calcium/creatinine) for the control, CH and CHH groups revealed a significant difference between CH (0.001 ± 0.0001, n = 7) and no difference between CHH (0.01 ± 0.002, n = 16) when compared with control (0.01 ± 0.001, n = 18). Again, it is likely that the low calcium content of the diet explains the significant reductions in renal calcium excretion and clearance in the CH group.

Colonic Oxalate Transport

Our previous studies have shown that enteric excretion of oxalate is induced in CRF rats and that this adaptation involves local ANG II mediation [4, 5]. These studies prompted the question of whether colonic transport of oxalate is altered in oxalate-loaded rats with or without renal dysfunction. The question of whether changes in oxalate fluxes, if any, involve ANG II was also addressed in the following experiments. In all of these experimental series using proximal and distal colon, the results presented were acquired during Per I. Results acquired during Per II showed there were no significant time-dependent changes in either the fluxes or the associated electrical parameters in any series over the duration of the two flux periods (data not shown).

Oxalate fluxes across the distal colon removed from rats in the three oxalate-loaded groups are compared to control fluxes in figure 2. The results show that control rats (including rats injected with saline) and CH rats, support a net absorptive flux of oxalate of comparable magnitude. In contrast, coordinated changes in the unidirectional fluxes of oxalate resulted in a significant net secretion of oxalate across colonic tissues removed from AH and CHH rats. These changes in oxalate transport were not accompanied by changes in Cl– transport and there were no alterations in the associated electrical characteristics.

Whereas the distal colon supports net oxalate and Cl– absorption, we have observed that the proximal colon supports a basal net secretory flux of both anions [6] and these results are confirmed here (fig. 3). It is also apparent that there are no significant alterations in either oxalate or Cl– transport across this segment in any of the treated groups.
Effects of Losartan on Colonic Anion Transport in CHH and AH Rats

As mentioned above, we have shown that net oxalate secretion in CRF distal colon is partly mediated by AT₁ receptor agonism based upon a sensitivity to losartan [4, 5]. We have also observed that basal oxalate secretion in the proximal colon of control rats is insensitive to losartan (data not shown). Consequently, in the next series, we examined the effects of losartan on oxalate secretion that was manifest only in CHH and AH distal colonic segment. As shown in figure 4, losartan addition, at a concentration (10⁻³ M) known to have a maximum inhibitory effect on Cl⁻ transport across this tissue preparation [12], had no significant effect on the net flux of oxalate in CHH despite a small but significant reduction in the secretory component (J_{s,m}^{OX}) of the net flux. These results suggest that net oxalate secretion stimulated in the CHH model is not primarily dependent upon AT₁ agonism. In contrast, net oxalate secretion in AH distal colon (fig. 5) was abolished by losartan addition, albeit a net absorptive flux was not fully restored as we have seen before in CRF rat models [4, 5]. The changes in Cl⁻ fluxes following losartan are entirely consistent with what we have previously observed in chloride-absorbing tissues, namely reductions in unidirectional fluxes with no significant alteration in the net flux [12]. Tissue conductance was not altered in any series.
Fig. 5. Effects of ANG II receptor antagonism on the unidirectional and net transepithelial fluxes of oxalate (A) and chloride (B) across isolated, short-circuited segments of the distal colon from the acute hyperoxaluria (AH, n = 8) rat model. For each tissue pair control fluxes (open boxes) were established in Per I after which losartan was added (10^{-3} M) to the serosal sides of the tissues. Period II (hatched boxes) represents flux measurements following losartan addition. The number of tissue pairs is represented by n. Error bars represent ± 1 SE of the mean and an asterisk indicates a significant difference from Per I as judged by Student’s t test with p \leq 0.05. Losartan addition did not significantly change Isc or GT in the AH rats from control values of 4.7 ± 0.4 μEq·cm^{-2}·h^{-1} and 11.4 ± 0.5 mS·cm^{-2}, respectively.}

Presented in figures 4 and 5 but the reduction noted in I_{sc} across CHH tissues (fig. 4, legend) suggests that losartan had an affect on the electrogenic movement of some ion, other than chloride.

Discussion

Previous studies have demonstrated that enteric secretion of oxalate provides an additional excretory route which becomes important when renal function declines [2, 3]. The present study addressed the question of how the renal and enteric routes of oxalate elimination are coordinated in rats following an oxalate load. In the following discussion it is important to note that the magnitude of the oxalate challenges presented to the kidneys of these rats was likely different among the experimental groups because the systemic oxalate load was derived in a different way in each model. For example, a calculation, based upon a minimum (10%) conversion of ethylene glycol to oxalate, would suggest that the oxalate load delivered to the CHH rat was at least 10-fold smaller than that delivered to the AH rat. We also acknowledge that the rate of diffusion of the load injected into the peritoneal cavity will impact the amount of oxalate challenging the kidneys at any one point in time. In the CH group, dietary oxalate absorption was undoubtedly a limiting factor in the magnitude of the oxalate load presented to the kidneys in this model. It was also apparent that this latter group sustained normal plasma oxalate levels and normal renal function with maximal urinary oxalate excretion at least for the duration of the study. Certainly, the time course of the various regimens differs and in this context we note that the AH group, in particular, is not a ‘steady-state’ model. There is also a temporal component that is associated with the duration of chronic hyperoxaluria, since we have observed that (CH) rats maintained on the dietary oxalate-loading regimen for an extended period (>3 weeks), exhibit a reduction in renal function [unpubl. data]. Renal function in ethylene glycol (0.75%)-treated rats can apparently remain unaltered for many weeks/months despite chronic hyperoxaluria [unpubl. data] and despite renal damage as indicated by significant lipid peroxide production as early as the 15th day of treatment [13].

Renal Handling of Oxalate

Although urinary oxalate excretion was increased in all oxalate-loaded groups, renal function (as judged by creatinine clearance and/or plasma creatinine) appeared to be reduced only in AH rats. The small but significant increase in plasma creatinine in this group cannot be explained by the degree of hyperoxaluria induced (9 times), given this was even greater in CHH rats (16 times) whose renal function was found to be normal. In addition, plasma oxalate in both groups was comparably elevated. One possible explanation may be that the acute oxalate load delivered intraperitoneally presents a larger oxalate challenge, and is immediately more damaging to renal function than a load derived gradually from systemic metabolism of ethylene glycol. Severe renal damage due to calcium oxalate crystallization has been well documented in this acute model using larger injected doses [14]. However, at this low dosage of 3 mg/100 g body weight, Khan et al. reported no evidence of renal damage

Renal and Intestinal Oxalate Excretion in Rats

Am J Nephrol 2003;23:18–26
as judged by urinary creatinine excretion and urinary enzyme markers 6 h after injection, but plasma creatinine concentrations were not determined. In another oxalate-loaded model, Kumar et al. [15] did report significant increases in urinary enzyme markers, indicative of renal injury, which was not accompanied by alterations in urinary creatinine excretion but, again, plasma creatinine concentrations were not reported. It is notable here that we have shown that creatinine clearance can be significantly reduced in rats with no significant change in urinary creatinine excretion achieved under ‘steady-state’ conditions [3, 12, 16].

Many studies examining renal excretion of oxalate by humans and animals have relied upon oxalate/creatinine clearance ratios to indicate either tubular secretion (clearance ratios >1) or tubular reabsorption (clearance ratios <1) of oxalate. Such studies have demonstrated that clearance ratios can range from mean values between 0.4 and 2.0 in healthy individuals as well as in stone-forming patients [17–27], with considerable overlap of values when all studies and both groups are considered. In general, the oxalate/creatinine ratio is elevated (ratio >3) in patients with primary hyperoxaluria [17, 18, 20] compared to controls. Studies that we have conducted, examining oxalate and creatinine clearances in humans [24, 28] and rats [3], have revealed oxalate/creatinine clearance ratios that are similar and <1 in controls of both species. These observations are confirmed in the present study for the controls. In addition, the capacity of the kidney to secrete oxalate was evident in all of the oxalate challenged groups here. The marked increase in both renal clearance (28 times) and the clearance ratio (19 times) in the CH group would suggest that the capacity of the healthy kidney to excrete oxalate is considerably greater than previously thought. Although a significant renal secretion of oxalate also occurred in the CHH rats, it is nonetheless curious that renal clearance of oxalate and the estimate of the secretory capacity (i.e. the oxalate/creatinine clearance ratio), are 4- and 5-fold less, respectively, in this group when compared to CH (these differences are statistically significant). The reason for this is unclear since renal function and the degree of hyperoxaluria are comparable in both groups. What is different between the two groups is plasma oxalate concentration which is normal in CH and elevated approximately 4-fold in CHH. A further difference between these groups is adaptive enteric oxalate secretion which is induced in the distal colon of CHH but not in CH rats, and this is discussed below.

**Calcium**

The oxalate-loading regimens used in the present study had no effect on plasma calcium homeostasis but there were some effects on renal calcium handling. It was expected to find that renal excretion and clearance of calcium were markedly reduced in the CH group, since the diet fed to these rats contained 0.01% calcium compared to a calcium content of 0.95% in the rat chow fed to the control, CHH and AH rats. This oxalate-supplemented diet was purposely designed with low calcium content to promote optimal bioavailability and oxalate absorption along the intestinal tract in order to induce hyperoxaluria. In contrast, the 50% reduction in renal excretion of calcium in the AH group was unexpected. While the reasons for this are unclear, one possibility may be that chelation of calcium and renal/extrarenal tissue deposition of calcium oxalate following the acute, massive oxalate load provide for a calcium sink thereby reducing urinary calcium excretion. Indeed, a cursory examination of the kidneys from these rats did reveal obvious deposition both externally and internally but we did not conduct an examination of any other organs or tissues. In contrast to these results in AH, we found that gradual oxalate loading via ethylene glycol treatment did not reduce urinary calcium excretion. In fact, the small but significant increase in renal clearance of calcium in these rats suggested a possible reduction in tubular calcium reabsorption, but, this was not reflected by a change in the mean clearance ratio (calcium/creatinine) of this group when compared to control.

**Enteric Oxalate Handling**

The results of the colonic transport studies indicate that hyperoxaluria, per se, does not appear to induce alterations in colonic oxalate handling. Colonic transport of oxalate in CH rats was normal despite significant chronic hyperoxaluria. The results do indicate that adaptive enteric oxalate secretion can be correlated with elevations in plasma oxalate concentrations in the absence of overt renal insufficiency. This latter point is important because we had concluded from our prior studies that the reversal in the direction of net oxalate transport across the distal colon, from absorption to secretion, was correlated with some degree of renal insufficiency [3–5]. Previously, we demonstrated that adaptive enteric oxalate secretion/excretion occurs in two different CRF rat models both exhibiting a 2-fold increase in plasma creatinine [3, 4]. Although urinary oxalate excretion was normal in one of these CRF models, both had elevated plasma oxalate concentrations. In the present study we found that colonic...
tissues from AH and CHH rats supported net oxalate secretion, both of these groups were hyperoxaluric with comparable hyperoxalemia, however, renal function was reduced in AH and normal in CHH. Thus, in the rat models examined that exhibit adaptive enteric oxalate secretion, they have in common an elevation in plasma oxalate. This observation is supported by results from Costello et al. [2] who showed that percent fecal 14C-oxalate excretion was directly correlated with elevations in plasma oxalate in CRF. Results from the latter study which followed the distribution of tracer also showed that the percent of 14C-oxalate excreted in feces can account for up to 40% of the tracer burden. Therefore, it is conceivable that enteric elimination of oxalate could be significantly in excess of 40% of the oxalate load following the regimens described here. On the basis of this and the flux studies conducted here, we suggest that significant enteric elimination of oxalate in CHH and AH rats accounts for the ~5-fold difference in renal oxalate secretory capacity (i.e. as judged by mean oxalate/creatinine clearance ratios) when compared to CH rats.

Effects of Losartan on Enteric Oxalate Secretion

While enteric oxalate secretion/excretion was induced in CHH and AH rats, it appeared that there were some mechanistic differences between the two groups in how this secretory process was mediated. Net secretion of oxalate across the distal colon of AH rats was significantly reduced by AT1 receptor antagonism (losartan) and it remained unaltered in CHH rats following the same maneuver. Clearly, losartan addition to isolated AH distal colon did not completely reverse net secretion of oxalate to a net absorptive flux as we have seen before in CRF rats [4, 5]. However, the significant reduction in both JOX and J0X in AH implicates the involvement of ANG II in mediating oxalate secretion. Although J0X was decreased in CHH distal colon, ANG II involvement in mediating enteric excretion of oxalate in CHH rats was not strongly indicated.

In summary, these results support the concept that oxalate elimination is balanced between the renal and excretory routes. We suggest that the capacity of the kidney to secrete oxalate may be considerably greater than previously thought. Several features of oxalate handling by the large intestine of oxalate-loaded rats emerged from this study: (1) The distal segment is the primary site for adaptive enteric oxalate elimination associated with elevations in plasma oxalate. (2) We observed, for the first time, that colonic oxalate and chloride secretion can be dissociated. (3) There are additional oxalate secretory pathways, independent of ANG II regulation and distinct from those electrogenic chloride secretory pathways previously implicated in oxalate secretion [7, 29].

Acknowledgements

This work was supported in part by a grant from the Oxalosis and Hyperoxaluria Foundation and in part by NIH (R01 DK56245).

References


