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ORIGINAL PAPER

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Gastrointestinal oxalic acid absorption in calcium-treated rats

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Abstract We studied whether urinary oxalate excretion after an acute oral load of oxalic acid is influenced by concomitant administration of calcium in rats. Male Wistar rats weighing approximately 180 g were divided into six groups of five animals each. After inducing anesthesia, the animals were orally (via a gastrostomy) given 110 μmol of oxalic acid along with 0, 27.5, 55, 110, or 220 μmol of calcium (0, 27.5, 55, 110, or 220 μmol Ca group, respectively). Saline was given to the control group instead of oxalic acid. Urine specimens were collected before administration and then at hourly intervals up to 5 h afterward. Urinary oxalate and citrate levels were measured by capillary electrophoresis, while urinary calcium, magnesium, and phosphorus levels were measured by ICP spectrophotometry. Urinary oxalate excretion peaked at 1 h after administration and was higher in the 0, 27.5, and 55 μmol Ca groups than in the control group. The urinary recovery of oxalate in these groups was 10–15%, while the recovery rate was less than 3% in other groups. Urinary Ca excretion showed no significant changes, either over time or between groups. Free oxalic acid is absorbed more readily from the gastrointestinal tract than calcium oxalate, while simultaneous administration of calcium appears to block intestinal oxalic acid absorption in a dose-dependent manner.

Keywords Oxalic acid · Calcium · Intestinal absorption · Bioavailability

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Introduction

Calcium oxalate stones are the most common urinary tract calculi. High levels of urinary oxalate have a significant influence on urinary calcium oxalate supersaturation and calcium oxalate crystal formation, thus being an important risk factor for calcium oxalate stones [1]. Urinary oxalate is derived from two sources: endogenous production in the liver accounts for approximately 80% of oxalate excreted in the urine, while dietary intake accounts for 10–20% [2]. It is now recognized, as reported by Holmes et al. [3] that depending on the dietary contents dietary intake of 150–250 mg oxalate can account for up to 40–50% of urinary oxalate. Dietary oxalate absorption has been reported to play a very important role in recurrent formation of calcium oxalate stones [4], and absorption from intestinal tract is promoted by an increased dietary intake of oxalate [5]. Oxalate is abundant in most green vegetables [6] and is either absorbed from the intestine, digested by oxalate-degrading bacteria in the colon [7,8], or excreted from the gastrointestinal system as insoluble salts after binding with calcium [9, 10].

It is generally accepted that calcium oxalate is less readily absorbed than soluble oxalate [3, 9, 10], so the bioavailability of dietary oxalic acid depends mainly on the calcium content in the diet [9]. Calcium salts, or dairy products containing calcium (especially high-calcium milk and low-fat milk) can decrease the gastrointestinal absorption of oxalate and its subsequent urinary excretion [3, 10–12]. However, Hanes et al. [13] have suggested that absorption of calcium oxalate does not require dissociation of this molecule.

Therefore, we studied urinary oxalate excretion after the acute oral administration of 110 μmol of oxalic acid with or without various doses of calcium (0–220 μmol) to clarify the influence of calcium itself on intestinal oxalic acid absorption, presuming that urinary oxalic acid excretion after oral intake may reflect the amount of absorbed oxalic acid.

Materials and methods

Male Wistar rats weighing approximately 180 g were acclimatized for 1 week at our University Animal Center and then were randomly divided into six groups (five experimental groups and a control group) of five animals each. All animals were fasted with free access to drinking water for 24 h before the experiment. After the rats were anesthetized with intraperitoneal urethane (0.12 g/100 g body weight), physiological saline was infused intravenously via femoral vein at a rate of 3–3.5 ml/h. After 2 h of hydration, a dose of 110 μmol of oxalic acid (1 ml) was given via a gastrostomy simultaneously with 1 ml of saline (0 μmol Ca group), 27.5 μmol of calcium (27.5 μmol Ca group), 55 μmol of calcium (55 μmol Ca group), 110 μmol of calcium (110 μmol Ca group), or 220 μmol of calcium (220 μmol Ca group). In the control group, physiological saline (2 ml) was given instead of oxalic acid and calcium.

Oxalic acid solution was prepared by dissolving 10 mg of oxalic acid dihydrate (molecular weight 126.07; Wako Pure Chemicals©, Osaka, Japan) in 1 ml of pure water, while the calcium solutions were prepared by dissolving the required amount of calcium chloride dihydrate (molecular weight 147.02; Wako Pure Chemicals©) in 1 ml of pure water.

Urinary bladder was exposed and urine specimens were collected by repeated bladder puncture using disposable needle and syringe at every hour just prior to administration and at hourly intervals up to 5 h afterward, and were immediately stored at −80°C until assay. Thawed urine was acidified to pH 2 or less with 6 N HCl, and filtered through a disposable 0.2 μm filter (Millex-LG syringe-driven unit, Millipore, Bedford, MA, USA). Then the specimens were diluted 20- to 40-fold with Milli-Q level pure water (Millipore Water Purification System) and injected into a capillary tube at 50 mbar (5,000 pa) for 4 s (approximately 20 nl), in order to measure urinary oxalate by capillary electrophoresis (Hewlett-Packard, Waldbronn, Germany) using a buffer (pH 7.7) for high performance capillary electrophoresis (HPCE) (Fluka, Switzerland). Urinary citrate was measured using an organic acid buffer (pH 5.6) for HPCE containing 5 mM 2,6-pyridinedicarboxylic acid with 0.5 mM cetyltrimethylammonium bromide (CTAB) (Hewlett-Packard, Waldbronn, Germany). Urine specimens were also diluted 200- to 400-fold with pure water to measure urinary calcium, magnesium, and phosphorus by inductively coupled plasma spectrophotometry (ICPS-7000, Shimadzu©, Kyoto, Japan).

The urinary levels of oxalate, citrate, calcium, magnesium, and phosphorus at 0 h were defined as the baseline excretion of each substance. The cumulative urinary recovery of the oxalate dose up to 5 h after administration was calculated in each experimental group by subtracting the baseline oxalate value.

Data were analyzed by one-way analysis of variance (ANOVA). Hourly urinary excretion and increments relative to the baseline value were compared over time using stratified multiple regression analysis, and were compared between groups using Student's t test with Bonferroni's correction. For all analyses, statistical significance was set at $P < 0.05$.

Results

Hourly urinary oxalate excretion peaked at 1 h after administration in the 0, 27.5, 55, and 110 μmol Ca groups (Table 1). It showed a significant increase from baseline (0 h) at 1–5 h in the 0 μmol Ca group, as well as at 2–4 h in the 27.5 and 55 μmol Ca groups. In addition, it was significantly higher from 1 to 5 h in the 0 μmol Ca group, at 2, 4, and 5 h in the 27.5 μmol Ca group, and at 2–5 h in the 55 μmol Ca group compared with the control group. It was also significantly higher in the 0 μmol Ca group than in 110 and 220 μmol Ca groups ($P < 0.05$). Hourly urinary oxalate excretion showed a peak at 1 h, and was not significantly higher from 1 to 5 h in the 110 μmol Ca group compared with that in the control group. Urinary oxalate did not increase at all in the 220 μmol Ca group and control group (Table 1).

Cumulative urinary oxalate excretion up to 5 h (mean ± SD) was 15.80 ± 5.21, 12.11 ± 8.64, 10.92 ± 7.40, 2.52 ± 4.32, and 0.05 ± 1.00% of the administered dose of oxalic acid in the 0, 27.5, 55, 110, and 220 μmol Ca groups, respectively (Fig. 1). It was significantly higher in the 0, 27.5, and 55 μmol Ca groups than in the control group ($P < 0.05$). Regression analysis showed a significant negative correlation between the dose of calcium ingested and urinary oxalate excretion ($Y = -0.0439X + 9.803$, $r = -0.4225$, $P = 0.02$).

Hourly urinary calcium excretion peaked at 2–3 h after dosing, but it was not significantly different from baseline (Table 1).

Discussion

Dietary oxalate is effectively absorbed at low intake levels of 10 mg/day, but oxalate absorption shows a limited increase at high intake levels that suggests saturation of oxalate transporters in the gut. A higher percentage of ingested oxalate is thought to be ionized and thus available for absorption at low intake levels than at higher intake levels, when a large fraction of dietary oxalate may be complexed and unavailable for absorption [14]. Their findings were compatible with our previous experimental result that urinary oxalate excretion increased in a dose-dependent fashion (5–20 mg) with a peak at 1 or 2 h after an oxalate load and that the maximum absorption rate was 5 μmol/h in rats [15]. In contrast, it has been reported that there was no apparent difference in the absorption or excretion of oxalate between groups administered either sodium oxalate (7.8 or 5 mmol) or calcium oxalate (6.8 or 5 mmol) [16]. The theory of Hanes et al. [13] that absorption of calcium

Table 1 Urinary oxalate and calcium excretion after an oxalic acid load with various doses of calcium (via a gastrostomy) in rats. In the groups receiving lower calcium doses, hourly urinary oxalate excretion showed a significant increase from baseline. Moreover, oxalate excretion was significantly higher in the low calcium groups than in the control group, high calcium group, and equimolar calcium group

Calcium treatment (μmol)	Oxalic acid treatment (μmol)	Urinary oxalate excretion (μmol)					
		0 h (baseline)	1 h	2 h	3 h	4 h	5 h
0	110	0.444 ± 0.108	4.62 ± 2.18[a–c]	4.21 ± 1.47[a–d]	3.77 ± 0.799[a–d]	2.76 ± 0.689[a–d]	2.66 ± 1.95[a–d]
27.5	110	0.438 ± 0.117	5.09 ± 4.97	3.43 ± 1.51[a–d]	2.30 ± 1.51[a]	2.11 ± 1.18[a,b]	1.36 ± 0.52[a–c]
55	110	0.4 ± 0.100	3.45 ± 3.34	3.22 ± 2.06[a–d]	2.52 ± 1.31[a–d]	1.84 ± 0.760[a–d]	1.89 ± 0.850[a–c]
110	110	0.376 ± 0.081	1.50 ± 2.05	0.926 ± 0.770	0.746 ± 0.738	0.67 ± 0.532	0.558 ± 0.373
220	110	0.372 ± 0.093	0.444 ± 0.222	0.452 ± 0.222	0.338 ± 0.198	0.35 ± 0.170	0.328 ± 0.373
0	0	0.356 ± 0.255	0.27 ± 0.076	0.286 ± 0.111	0.158 ± 0.124	0.204 ± 0.119	0.172 ± 0.052
Calcium treatment (μmol)	Oxalic acid treatment (μmol)	Urinary calcium excretion (μmol)					
		0 h (baseline)	1 h	2 h	3 h	4 h	5 h
0	110	0.286 ± 0.047	0.292 ± 0.092	0.348 ± 0.166	0.51 ± 0.203	0.482 ± 0.165	0.522 ± 0.099
27.5	110	0.338 ± 0.097	0.296 ± 0.036	0.502 ± 0.240	0.412 ± 0.115	0.456 ± 0.105	0.466 ± 0.187
55	110	0.322 ± 0.249	0.372 ± 0.187	0.386 ± 0.261	0.52 ± 0.245	0.602 ± 0.338	0.836 ± 0.644
110	110	0.274 ± 0.098	0.3 ± 0.116	0.502 ± 0.264	0.858 ± 0.559	0.708 ± 0.672	0.638 ± 0.608
220	110	0.298 ± 0.114	0.284 ± 0.072	0.52 ± 0.382	0.534 ± 0.171	0.578 ± 0.302	0.492 ± 0.249
0	0	0.294 ± 0.155	0.51 ± 0.447	0.824 ± 0.398	0.466 ± 0.143	0.936 ± 0.522	1.12 ± 0.622

[a] vs. baseline
[b] vs. control group
[c] vs. 220 μmol Ca group
[d] vs. 110 μmol Ca group $P < 0.05$

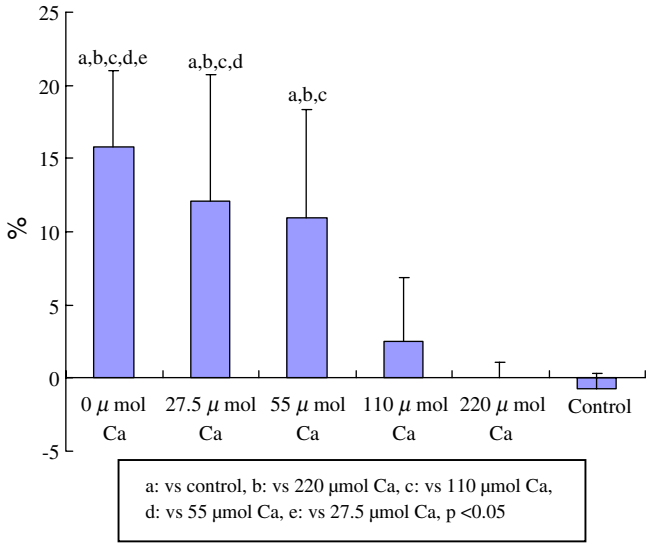

Fig. 1 Mean cumulative urinary oxalate excretion after an acute oxalic acid load with various doses of calcium (via a gastrostomy) in rats ($n = 5$ per group)

oxalate does not require prior dissociation in rats is also controversial. Accordingly, we tested their hypothesis and showed that soluble oxalate is mainly absorbed because calcium oxalate was poorly absorbed, at least from the upper gastrointestinal tract [10]. The present study also confirmed that dietary calcium inhibits oxalate absorption from the gut, suggesting that free oxalate absorption is much greater than that of calcium oxalate.

A low dietary calcium intake (250 or 391 mg/day) has been reported to increase urinary oxalate excretion (7–10 mg/day, 4.4 Ox/Cr mg/g) due to inadequate binding of oxalate by calcium in the intestinal tract [3, 16]. A high calcium intake (3,858 mg/day) combined with an oxalate-rich diet (2,220 mg) prevents dietary hyperoxaluria in healthy subjects [17]. Adding calcium to a regular diet induces a significant decrease of urinary oxalate excretion in persons who are normocalciuric and patients with dietary hypercalciuria [18]. In the present study, the increase of urinary oxalate excretion was inversely correlated with the calcium dose, presumably depending on the amount of free oxalic acid, not bound to calcium in the gut, which suggests that urinary oxalate excretion depends on the amount of calcium available in the gut lumen. This showed the pure effect of various relative levels of dietary calcium (from 2-fold higher to lower than the oxalate dose) on the absorption of oxalic acid. Urinary oxalic acid excretion was similar after administration of oxalate and equimolar disodium oxalate, while administration of calcium oxalate only caused a small increase in urinary excretion [10]. Undoubtedly, ingested calcium binds to ionized oxalate and affects intestinal oxalate absorption [19], as we have also shown that calcium salts or dairy products containing calcium (especially high-calcium milk and low-fat milk) can decrease the gastrointestinal absorption and subsequent urinary excretion of oxalate [10, 20]. Moreover, several dietary factors besides calcium including magnesium and fatty acids might affect intestinal oxalic acid absorption. The influence of low luminal calcium levels on oxalate absorption may also be mediated through an effect on tight junction permeability [21]. Reciprocal changes of tight junction permeability (reflected by transepithelial conductance) with changes of the calcium concentration have been demonstrated in rat colonic epithelium [22]. Taken together, a low level of intestinal calcium relative to that of oxalic acid will allow oxalic acid to remain free in the gut lumen and thus be absorbed from the intestine. Increased oxalic acid absorption associated with a reduced calcium intake could be magnified when dietary oxalic acid does not decrease in parallel. We want to emphasize that ingested calcium has an important influence on intestinal oxalate absorption.

Epidemiological studies show that restriction of calcium intake leads to an increase of urinary oxalate excretion and recurrence of calcium oxalate stone [23]. This finding is compatible with our previous studies in which rats fed various doses of calcium and oxalic acid for 4 weeks developed hyperoxaluria and calcium oxalate stones in a dietary Ox/Ca ratio-dependent manner (> 1 mol/mol) [9]. Thus, the availability of oxalic acid for absorption from the gut increases under conditions that reduce calcium availability in the intestinal lumen, such as restriction of calcium intake [3, 19, 23], absorptive hypercalciuria [24], or administration of calcium binders like cellulose phosphate (which have been used to treat hypercalciuric stone formers) [25]. In addition, luminal fatty acids could constitute another mechanism for the hyperabsorption of oxalate associated with various malabsorptive disorders because these fatty acids tend to form complexes with calcium [26].

Since oxalate absorption peaks within 4 h after ingestion, the small intestine is thought to be a major site of oxalate absorption in normal individuals [27]. Urinary oxalate excretion was found to peak at 1 h after an oxalate load and decreased 2–3 h later in rats. This also confirmed that the upper gastrointestinal tract is a major site of oxalate absorption. However, absorption from the upper and lower intestine accounted for 6% (18 min) and 16% (140 min) of a dose of 0.375 mmol of 14C-oxalic acid in rats [28]. Traditionally, the early appearance of an orally administered nutrient in the blood or urine has been interpreted to reflect absorption by the small intestine. In addition, the stomach has been suggested as a new and critical site for oxalate absorption because of the prompt increase of urinary oxalate excretion after oxalate loading via a gastric tube and blockage of the gastric outlet with an intrapyloric balloon [29]. It is possible that both the stomach and the small intestine might play an important role in oxalate absorption in rats because the gastric-ileal transit time after an ordinary meal is approximately 3–4 h.

In conclusion, there was no significant increase of urinary oxalate excretion in rats when dietary calcium

intake was higher than oxalate intake (calcium dose > oxalate dose). Therefore, oxalic acid may need to be soluble for absorption and calcium may inhibit oxalate from being absorbed. Appropriate dietary intake of calcium may be a better therapeutic strategy for decreasing oxalate absorption not only for recurrent calcium oxalate stone formers, but also for enteric hyperoxaluric calcium nephrolithiasis. However, the bioavailability of dietary oxalate in rats may also depend on the relative intestinal levels of magnesium, dietary fiber, bile salts, and fatty acids, along with the intestinal calcium level [24, 30, 31]. Therefore, further comprehensive studies that assess all of these substances are warranted.

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