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Oxalate and Sucralose Absorption in Idiopathic Calcium Oxalate Stone Formers

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Abstract

Objectives—Oxalate has been hypothesized to undergo absorption in the large and small intestine by both paracellular and transepithelial transport. Sucralose is a chlorinated sugar that is absorbed by paracellular mechanisms. This study's objective was to better understand intestinal oxalate transport by correlating oxalate and sucralose absorption in idiopathic calcium oxalate stone formers.

Methods—Idiopathic calcium oxalate stone formers were recruited to provide urine specimens on both a self-selected diet and following a meal containing 90 mg of ¹³C₂ oxalate and 5 grams of sucralose, and a stool sample for determination of *Oxalobacter formigenes* colonization. The 24 hour urine collections were fractionated into the first 6 hours and the subsequent 18 hours. Sucralose and oxalate excretion were measured during these periods and used to estimate absorption.

Results—A total of 38 subjects were evaluated. The majority of both the ¹³C₂ oxalate and sucralose absorption occurred within the 0-6 hour collection. The ¹³C₂ oxalate and sucralose absorptions were significantly correlated at the 0-6 hour, the 6-24 hour, and the total 24 hour time periods (p<0.04). All five oxalate hyperabsorbers (> 15% absorption) also absorbed significantly more sucralose during the 0-6 hour and whole 24 hour time points (p<0.04). *Oxalobacter formigenes* colonization did not significantly alter oxalate absorption.

Conclusion—The results suggest that the majority of oxalate is absorbed in the proximal portion of the gastrointestinal tract and that paracellular transport is involved. Augmented paracellular transport, as evidenced by increased sucralose absorption, may also influence oxalate absorption.

Keywords

Urolithiasis; oxalate; intestinal permeability; sucralose; absorption

Introduction

In typical Western diets, ~ 100 – 300 mg of oxalate is consumed daily and 5 – 10% is absorbed in the intestine and excreted in urine ^{1, 2}. The amount absorbed has been reported to be higher in stone formers than non-stone formers and to increase significantly following

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certain types of bariatric surgery or with the development of intestinal disease^{3, 4}. This oxalate contributes to the formation of calcium oxalate kidney stones as even small increases in urinary excretion are associated with an increased stone risk⁵.

Oxalate is thought to be absorbed in the intestine by both paracellular and transepithelial transport⁶. It has been hypothesized that paracellular transport is more prominent in the small intestine than the large intestine as junctional resistance is lower and luminal oxalate content is higher⁶. Transepithelial transport is regulated by the SLC26 family of anion transporters and knock out mouse models have demonstrated the influence of 3 such transporters in these processes, SLC26A6 (PAT1), SLC26A3 (DRA) and SLC26A1 (SAT1)⁶⁻⁹. Oxalate loading studies in humans have demonstrated that a significant amount of oxalate is absorbed in the first 2-6 hours after its administration^{4, 10} suggesting that a significant amount of oxalate is absorbed in the small intestine. The slow decline in urinary oxalate excretion on an oxalate-free diet shows that absorption from the large intestine is also significant¹.

Sucralose, a chlorinated sugar utilized as an artificial sweetener, is not transported across the gut epithelium but moves via paracellular pathways. It is not metabolized by bacteria in the colon and after absorption it is excreted unchanged in urine. These properties have allowed it to be used to estimate whole gut permeability and as an index of intestinal paracellular transport¹¹. We have compared both oxalate and sucralose absorption in calcium oxalate stone formers in an effort to better understand mechanisms of intestinal oxalate transport in this cohort.

Methods

This study was approved by the Institutional Review Board of Wake Forest University School of Medicine. Fifty-nine subjects were recruited from April 2007 to July 2010 with 38 subjects completing the study. Inclusion criteria required subjects to be competent adults, 18-64 years of age, with a history of calcium oxalate kidney stones, defined by at least one stone analysis demonstrating > 50 % calcium oxalate. Exclusionary criteria included a BMI > 35, and a history of any hepatic, renal, bowel or endocrine disease (i.e. pregnancy, primary hyperparathyroidism, renal tubular acidosis, enteric hyperoxaluria, primary hyperoxaluria, cystinuria, chronic kidney disease) or any other condition that may influence the absorption, transport or urinary excretion of ions. The subjects did not consume vitamins or supplements during the day of the study.

The subjects initially collected two 24 hour urine specimens while consuming self selected diets and provided a stool sample to determine colonization with *Oxalobacter formigenes* (OxF) using an established culture test¹². After an overnight fast, they drank 500 ml of distilled water and voided at 8:00 AM (initial void). They then consumed a sausage biscuit, orange juice and 90 mg of ¹³C₂-oxalate (Cambridge Isotopes Laboratories) and 5 grams of sucralose (McNeil Nutritionals LLC, Ft Washington, PA) dissolved in 100 ml of distilled water. They then consumed self selected diets except for limiting dairy products and high oxalate containing foods for lunch and dinner. Urine was collected for 24 hours after the initial void but fractionated into the first 6 hours and the subsequent 18 hours.

Creatinine was measured on a Beckman C5E Analyzer. Total oxalate was measured using a kit provided by Trinity Biotech (St. Louis, MO). Reagent-Free ion chromatography coupled with negative ion electrospray mass spectrometry (RFIC-MS) (Dionex Corp.) was used to measure ¹³C₂-oxalate. A Thermo-Finnigan MSQTM ELMO single quadrupole mass spectrometer that is specifically designed for the analysis of low molecular weight ions was used for mass determinations. The IC portion of the IC/MS consisted of an ED50

conductivity detector, a GS50 gradient pump, an AS50 refrigerated autosampler, an EG50 potassium hydroxide gradient generator, and an AS50 thermal compartment containing an AS11-HC, 2×150 mm, anion exchange column at a controlled temperature of 30°C and an ASRS 300 2mm suppressor. A gradient of KOH from 0.5 to 80 mM over 60 min at a flow rate of 0.4 ml min^{-1} was used to separate anions in samples. The relative abundance of specific anions was determined by selected-ion monitoring (SIM) at the following mass/charge ratios: oxalate (SIM89) and $^{13}\text{C}_2$ -oxalate (SIM91). The enrichment of $^{13}\text{C}_2$ -oxalate in samples was determined from curves prepared using known amounts of $^{13}\text{C}_2$ -oxalate producing 0% to 40% enrichment.

Urinary sucralose excretion was measured using high performance anion-exchange chromatography with pulsed amperometric detection as described @ Dionex.com (Dionex application update 151). A CarboPacTM PA20 column, $3\text{mm} \times 150\text{mm}$, with 100mM sodium hydroxide/60mM sodium acetate as the eluent at a flow rate of 0.5ml/min was used to measure sucralose in urine samples. With this method, the limit of detection, defined as the mean blank signal plus $3 \times$ Standard Deviation (SD), and the limit of quantitation, defined as the mean blank signal plus $10 \times$ SD, were $0.07 \mu\text{M}$ and $0.16\mu\text{M}$, respectively. With each batch analysis, a urine control was run and the mean and SD sucralose level was $136.8 \pm 2.5 \mu\text{M}$, giving an inter-assay variability of 1.9%.

Experimental results were analyzed by a Student's t-test and correlation coefficients were determined by linear regression using Graph Pad software. P values < 0.05 were considered statistically significant. Data is expressed as mean $\pm$ SD.

Results

Thirty-eight subjects, 10 females and 28 males, completed this study. The mean age was $49 \text{ years} \pm 13 \text{ years}$. The mean urinary oxalate excretion measured in the 2 collections on self selected diets was $33.6 \pm 9.3 \text{ mg}$ and 10/38 (26%) had hyperoxaluria, defined as a mean excretion above 40 mg. Six (15.8%) of the subjects were colonized with *O. formigenes* (OxF). The mean 24 hour urinary oxalate excretion of the OxF positive cohort on self selected diets was lower ($29.4 \pm 7.0 \text{ mg}$) than those not colonized ($34.4 \pm 9.6 \text{ mg}$), but was not statistically different ($p = 0.24$).

The mean 24 hour $^{13}\text{C}_2$ -oxalate absorption was $8.7 \pm 5.9 \%$ for the whole group and the distribution is shown in Fig. 1A. Five subjects (13.2 %) hyperabsorbed oxalate with a value $> 15 \%$ which was previously used to define hyperabsorption by Voss et al ⁴. None of the hyperabsorbers were colonized with OxF. Furthermore, absorption was not statistically different in OxF colonized ($5.2 \pm 2.2 \%$) and non-colonized ($9.3 \pm 6.2 \%$) subjects ($p = 0.13$). Consistent with other studies, the bulk of the $^{13}\text{C}_2$ -oxalate absorption, 67%, occurred in the first 6 hrs after the load was administered (Fig. 2A) ⁴. This may represent predominantly absorption in the small intestine.

The mean 24 hour sucralose absorption in the entire group was $3.2 \pm 1.2 \%$ and its distribution in the cohort is shown in Fig. 1B. Similar to oxalate absorption, 58% of the sucralose absorption occurred during the first 6 hours (Fig. 2B). There were significant correlations between $^{13}\text{C}_2$ -oxalate absorption and sucralose absorptions for the 0 - 6 hour (Fig 3A), the 6 - 24 hour (Fig. 3B) and total 24 hour time periods (Fig. 3C).

There was a significant correlation between 24 hour $^{13}\text{C}_2$ -oxalate absorption and self-selected 24 hour urinary oxalate excretion ($r = 0.319$, $p = 0.006$) (Fig. 4). However, the 24 hour urine oxalate excretion of the oxalate hyperabsorbers on self selected diets was not significantly different than observed with the normo-absorbers, mean 39 mg versus 33 mg ($p = 0.25$). Oxalate hyperabsorbers ($n=5$) absorbed significantly more sucralose than normo-

absorbers during the whole 24 hour period; 4.3 % vs 3.1 % ($p = 0.034$) and during the 0-6 hour period; 2.5 % versus 1.8% ($p = 0.037$). However, it was not significant during the 6-24 hour collection period; 1.8% versus 1.3% ($p = 0.11$).

Discussion

Oxalate is a major constituent of the most common type of kidney stone; that composed of calcium oxalate. The importance of urine oxalate excretion was reported by Curhan and Taylor who demonstrated in 3 large cohorts that oxalate excretion was positively correlated with the risk of developing a kidney stone⁵. This association was even seen at levels of oxalate excretion considered to be within the normal range. At one time almost all oxalate in urine was thought to be derived from endogenous oxalate synthesis, there is now compelling evidence that a significant amount is derived from dietary sources. With a normal oxalate intake, approximately 40% of urinary oxalate is derived from the diet¹. Indirect evidence for this relationship was provided by Lieske and colleagues who showed that calcium oxalate stone formers with idiopathic hyperoxaluria reduced oxalate excretion by 36% when consuming a low oxalate diet¹³.

We observed a mean oxalate absorption of $8.7\% \pm 5.9\%$ in our study which is within the range reported by other groups using ^{13}C -oxalate loading. Hesse and colleagues reported that the mean oxalate absorption using this approach in 70 recurrent calcium oxalate stone formers was $9.2 \pm 5.1\%$ ³. In a subsequent study of 120 such subjects, they reported a somewhat higher mean absorption of $10.2 \pm 5.2\%$ ⁴. We detected a sub-group of stone formers who hyperabsorbed oxalate as defined by an absorption of greater than 15% as suggested by Voss et al⁴. Five subjects (13.2%) met this criterion, within the range previously reported by others. Hesse et al reported that 6/70 (8.6%) calcium oxalate stone formers hyperabsorbed in one study and 19/120 (15.8%) in a later study^{3,4}. In keeping with the study by Voss et. al., we observed a significant correlation between $^{13}\text{C}_2$ -oxalate absorption and urinary oxalate excretion.

Gastrointestinal transport is dependent on a number of factors including gender, food composition, medications, and method used to measure transit time¹⁴. However, it is generally accepted that small intestinal transport occurs within six hours of a meal with colonic transport requiring significantly more time. In our study, the majority of oxalate (67%) was absorbed in the first 6 hours after the ^{13}C -oxalate load. This suggests that a significant amount of the oxalate is absorbed proximal to the colon based on known GI transport times¹⁵. Similarly, Voss et al reported in 8 healthy adult subjects that 85% of the oxalate absorption occurred within the first 6 hours⁴. Chai reported that 83 % of the oxalate was absorbed in a group of 14 normal subjects and calcium oxalate stone formers during this same interval¹⁶.

Our results demonstrate significant correlations between sucralose and oxalate absorption for the whole 24 hour period and the two segmented collections. Sucralose is an artificial sweetener which is formed by the chlorination of sucrose and is resistant to degradation by bacteria in the colon. There is limited gut absorption and therefore what is transported is attributed to paracellular uptake. This property forms the basis of the use of its absorption as an index of whole-gut permeability¹¹. The mean 3.2% 24 hour sucralose absorption in our study is consistent with that published by other groups in adults without bowel disease. Farhadi and associates in 2 studies reported values of 2.32% and 2.49 %^{17,18}. Due to the significant absorption of sucralose in both the 0 – 6 and 6 – 24 hr periods, our results suggest that paracellular transport of oxalate occurs throughout the gastrointestinal tract. The absorption of the majority of oxalate and sucralose during the first 6 hours is consistent with

this absorption occurring in the small bowel. A higher paracellular transport in this region of the bowel is expected due to its low junctional resistance ¹⁹.

Colonization with OxF has been demonstrated to reduce the risk of recurrent calcium oxalate stone formation by 70 % ²⁰. None of the oxalate hyperabsorbers were colonized with OxF. A statistically significant relationship between colonization and oxalate hyperabsorption was not observed, but no conclusions can be reached due to the small number colonized. Further studies are required to determine the impact of OxF colonization on oxalate hyperabsorption. The hyperabsorbers did have a significantly higher 24 hour % sucralose absorption than the normo-absorbers. When the results were analyzed according to the collection period, a significant difference was observed only in the 0 - 6 hour period. This suggests that paracellular oxalate transport in the small intestine may contribute to oxalate hyperabsorption.

The apparent role of paracellular transport in oxalate absorption highlights the need for a better understanding of junctional permeability and function. Junctional complexes have a complex ultrastructure and may be comprised of such proteins as cadherin, catenin, occluding and claudin ²¹. Their permeability may not only be affected by these proteins, but also by the intestinal environment.

Transepithelial transport of oxalate is also important. Insight into this process has been provided by the study of the SLC26 family of anion transporters. Knock out mouse models have demonstrated the influence of 3 such transporters, SLC26A6 (PAT1), SLC26A3 (DRA), SLC26A1 (SAT1) on intestinal oxalate transport ⁶⁻⁹. Further investigations are required to better define both the transepithelial and paracellular transport of this anion. Better understanding of the mechanisms involved may lead to future therapeutic interventions.

We recognize that our study has certain limitations. Subjects were not placed on controlled diets which may have contributed to variability. A comparison with a normal age matched population was not undertaken and it is possible that the responses might be different in this cohort. In addition, others measures of gut permeability which could define regional gut differences were not utilized ²². This study assessed the absorption of soluble oxalate and we recognize that this form of oxalate in food varies, and thus could influence the dynamics of the absorption of food-derived oxalate.

Conclusions

Our study suggests that the majority of soluble oxalate is absorbed in the proximal portion of the gastrointestinal tract and that paracellular transport plays a role in its uptake. In addition, augmented paracellular transport, as evidenced by increased sucralose absorption, may influence oxalate hyperabsorption. This result requires further investigation to determine whether an inherent or acquired increase in gut permeability plays a role in oxalate hyperabsorption.

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Figure 1. Distribution of $^{13}\text{C}_2$ oxalate (Panel A) and sucralose (Panel B) absorption in 38 calcium oxalate stone formers.

Figure 2. $^{13}\text{C}_2$ oxalate (Panel A) and sucralose (Panel B) absorption over time in 38 calcium oxalate stone formers.

Figure 3.

Correlation of $^{13}\text{C}_2$ -oxalate absorption with sucralose absorption in 38 calcium oxalate stone formers. **A.** $^{13}\text{C}_2$ -oxalate absorption versus sucralose absorption 6 hours after $^{13}\text{C}_2$ -oxalate/sucralose load. **B.** $^{13}\text{C}_2$ -oxalate absorption versus sucralose absorption between 6 and 24 hours after the $^{13}\text{C}_2$ -oxalate/sucralose load. **C.** Total 24 hour $^{13}\text{C}_2$ -oxalate absorption versus sucralose absorption following the $^{13}\text{C}_2$ -oxalate/sucralose load.

Figure 4. Correlation of $^{13}\text{C}_2$ -oxalate absorption with urinary oxalate excretion in 38 calcium oxalate stone formers. Each data point represents mean $\pm$ SD of two 24 hour urines collected on self-selected diets.