Kidney360

A Prospective, Double-Blind, Randomized, Placebo-Controlled, Crossover Study Using an Orally Administered Oxalate Decarboxylase (OxDC)

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Abstract

Background Hyperoxaluria is typically associated with excessive oxalate intake in the diet, decreased dietary calcium, hyperabsorption of oxalate, or increased endogenous production of oxalate. The disorder spectrum extends from recurrent kidney stones to ESKD. This clinical trial sought to evaluate the effectiveness of an acid stable oxalate decarboxylase (OxDC) to reduce urinary oxalate in healthy subjects on a high-oxalate diet.

Methods In this prospective, double-blind, randomized, placebo-controlled, crossover clinical trial, 33 healthy volunteers were randomized into two crossover sequences separated by a 2-day washout period. A controlled high-oxalate diet (750–800 mg oxalate, 500–550 mg calcium daily) was utilized, and six 24-hour urine collections were measured. Subjects were given approximately 1000 U (micromoles per minute per milligram) of OxDC or placebo with meals three times daily during the 4 days of treatment.

Results Urinary oxalate significantly decreased with OxDC treatment. The baseline corrected within-subject mean reduction in 24-hour urinary excretion (after OxDC dosing versus high-oxalate baseline preceding treatment) was 12.5 mg or 29% ($P<0.001$). OxDC treatment was effective (>5% reduction) in 31 of 33 subjects (94%). Compared with placebo, OxDC produced a 24% reduction ($P<0.001$) in 24-hour oxalate excretion. Other urinary parameters (creatinine, uric acid, citrate, magnesium, calcium) were not affected by OxDC. No serious adverse events and no product-related adverse events occurred.

Conclusions An orally administered OxDC is capable of significantly reducing urinary oxalate levels in healthy volunteers on a high-oxalate diet without affecting creatinine clearance, urine creatinine, or other solutes related to supersaturation of calcium oxalate.

Clinical Trial registry name and registration number: Evaluation of Nephure, and the Reduction of Dietary Oxalate, in Healthy Volunteers, NCT03661216

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Introduction

Hyperoxaluria occurs when there is an excessive urinary excretion of oxalate. There are different forms of hyperoxaluria: idiopathic, enteric, and primary. Idiopathic hyperoxaluria is the most common form of hyperoxaluria, and it may be associated with excessive oxalate intake in the diet or decreased intake of dietary calcium or from increased endogenous production due to increased ingestion of oxalate precursors (1). Enteric hyperoxaluria results from malabsorptive disorders resulting from surgery (*e.g.*, small bowel resection or bariatric surgery) or inflammatory bowel disease, leading to increased oxalate absorption. Primary hyperoxaluria (types 1–3) is the result of inherited autosomal recessive disorder of glyoxylate metabolism (1). Currently, there is no established therapy for the reduction of urinary oxalate excretion in patients with calcium oxalate stones and idiopathic hyperoxaluria (2). Following confirmation of hyperoxaluria, patients are recommended to reduce intake of high-oxalate foods and to maintain a normal intake of calcium (3).

A proprietary oxalate decarboxylase (OxDC), has been developed as a food ingredient to reduce/remove both soluble and insoluble oxalate from a variety of foods and beverages (4). OxDC has the ability to degrade oxalate in food over a wide pH range, including

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the acidic pH of the stomach. This study considers the extent to which OxDC versus placebo reduces urinary oxalate excretion in healthy subjects who are provided a controlled high-oxalate diet.

Materials and Methods

Study Design

Bio-Kinetic Clinical Applications Institutional Review Board (Springfield, MO) approval was obtained prior to study initiation (Institutional Review Board Approval Letter 07/11/2018) and registered in clinicaltrials.gov (NCT03661216; date of registration: September 7, 2018). All subjects provided written informed consent with guarantee of confidentiality.

This was a prospective, double-blind, randomized, placebo-controlled, crossover study conducted in a confined clinical setting (Bio-Kinetic Clinical Applications, LLC, Springfield, MO) for 9 days under Good Clinical Practices in accordance with Food and Drug Administration Guidelines and the Declaration of Helsinki. Study recruitment initiated July 2018, and last subject's last visit was conducted in early August 2018. Following screening and baseline evaluations, all subjects were placed on a 4-day controlled high-oxalate diet plan. The controlled diet consisted of standard Western meals with added spinach and rhubarb to provide a high oxalate intake (750–800 mg/d) and low calcium intake (500–550 mg/d). Subjects started the controlled diet on day −2 (equilibrium period). Block randomization was implemented, with blinded randomization cards assigning into two different crossover sequences that included a placebo or OxDC on day 1. Blinded random allocation sequences and assignments were generated by DynaStat Consulting, LLC. Subjects were enrolled by Bio-Kinetic Clinical Applications, LLC. Subjects in each treatment sequence were administered either OxDC or placebo for 2 days (days 1 and 2). The 4-day controlled diet meal plan started over on day 3, the start of the 2-day washout phase (Figure 1). No food or drink from outside sources was permitted. Subjects ingested only what was provided but were allowed additional water between meals. Subjects were given approximately 1000 U (micromoles per minute per milligram) of OxDC or placebo with meals three times per day during the four treatment days. Study product was suspended in approximately 8 ounces of water prior to dosing by study staff. Product (OxDC and placebo) was provided to study staff in white sachets of 3 g each, identical in size, color, appearance, and weight.

The OxDC enzyme originates from *Synechoccus elongates* (PCC6301) and is expressed in *Escherichia coli*. The OxDC is a highly soluble, acid-resistant, manganese-containing enzyme with high catalytic efficiency, converting oxalate to formate and carbon dioxide. The purified enzyme is dried, mixed with maltodextrin, and filled into sachets to a unit level of approximately 1000 U/sachet. One unit of activity is the conversion of 1 μmol of oxalate to formate per minute per milligram of enzyme at 37°C and pH 3.

Study Population

All 33 subjects had the following entry criteria: age of 18–55 years, body mass index of 18.5–29.9 kg/m^2, eGFR of ≥60 ml/min per 1.73 m^2, urinary oxalate ≤40.5 mg/24 h, and urinary uric acid <750 mg/24 h. Men and nonpregnant women who were nonsmokers for 3 months at the time of screening and throughout the study were enrolled. Subjects were in good health as determined by complete physical examination, medical history, vital signs, and laboratory tests. Subjects were able to understand the nature of the study and comply with its requirements and restrictions, including completion of 24-hour urine collections. Subjects were able to comply with all dietary expectations and fluid intake. Women agreed to use an acceptable form of birth control from screening through the duration of the study.

Urine Analyses

The Mayo Clinic laboratory in Rochester, Minnesota analyzed and tested the 24-hour urine oxalate, citrate, and uric acid. Oxalate concentration was measured *via* a continuous flow assay using immobilized oxalate oxidase and

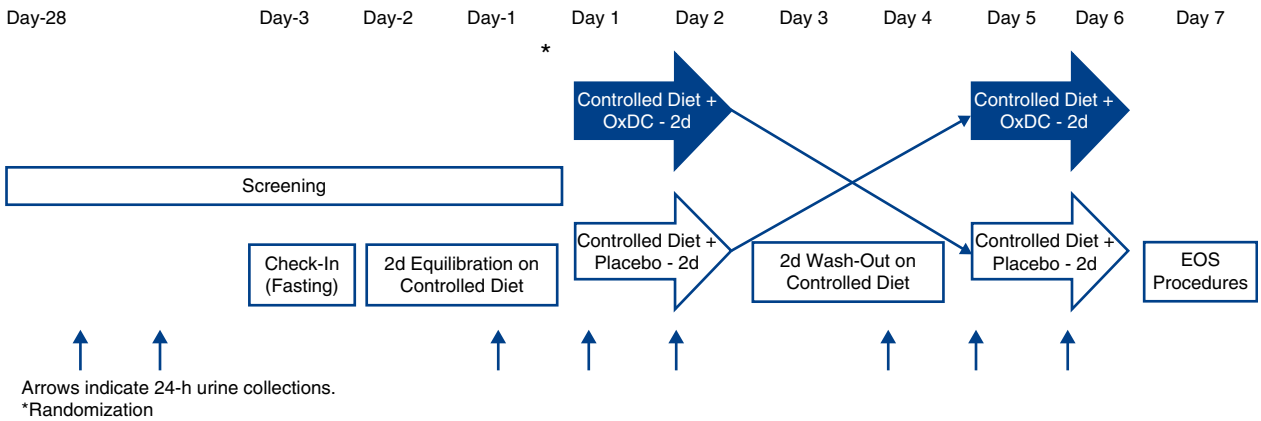

Figure 1. | **Study design.** The study included a screening period that began 28 days before randomization of subjects occurred, in which two 24-hour urine collections were performed. Following screening, subjects conducted six additional 24-hour urine collections (with collection start time on days −1, 1, and 2 and days 4, 5, and 6). The vertical arrows signify when the urine collections occurred. Sequence 1 of the study includes days 1 and 2. Sequence 2 consists of days 5 and 6. EOS, end of study.

peroxidase. Citrate concentration was obtained from a reaction involving citrate lyase and malate dehydrogenase measuring the disappearance of the light-absorbing NADH. Uric acid was measured *via* an enzymatic reaction involving uricase, peroxidase, and a color reagent, where intensity of color formation is proportional to uric acid concentration. Urinary calcium, magnesium, and creatinine were tested at the Mercy Hospital Springfield Laboratory in Springfield, Missouri using the Roche/Hitachi Cobas series analyzers.

No preservatives were used during urine collection. Urine collections were in control of clinic staff and kept refrigerated at all times. The 24-hour urine collections were mixed for 5 seconds immediately before any processing. Collection processing time was limited to 30 minutes, and aliquots intended for oxalate and citrate testing were acidified to pH<1.5 to calcium oxalate solubility. Urine collections during screening were collected by the participants in their own home or work environment.

Safety Analyses

The analysis of safety was performed by monitoring the incidence of treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), and adverse events associated with changes in laboratory values, vital signs, and physical examinations at screening and end of study.

Statistical Analyses

The sample size was calculated to detect a mean urinary oxalate (Uox) difference of 5 mg/24 h when comparing OxDC or placebo. The estimated sample size was 14 subjects to achieve a power of 90% and significance level of 0.05 (two sided) using the paired *t* test.

The end points are the mean difference of 24-hour Uox excretion between the OxDC and placebo groups and the baseline corrected within-subject difference in mean 24-hour Uox excretion (mean excretion while on OxDC versus mean excretion while on placebo per subject). The within-subject difference in mean 24-hour Uox excretion was tested by paired *t* test or Wilcoxon signed-rank test at the significance level of 0.05. Statistical analyses were performed using Statistical Analysis System (release 9.3 or higher).

Baseline is defined as the Uox (milligrams per 24 hours) for the high-oxalate diet period preceding treatment (days −1 and 4). All 33 subjects originally assigned were included in the final analysis. In analysis considering day-to-day variation, 31 subjects were included. Subjects, investigators, evaluators, and analysts were blinded, and all of the authors remained blinded until study completion.

Table 1. Baseline subject characteristics

Parameters	Value
Age, yr	36.2±12.27
Height, cm	169.3±8.30
Weight, kg	71.18±11.937
Sex, *n* (%)	
Men	17 (51.52)
Women	16 (48.48)
Race, *n* (%)	
White	29 (87.88)
Black	3 (9.09)
Other	1 (3.03)
eGFR, ml/min per 1.73 m^2	96.8±18.07

Values shown are the mean ± SD for the listed parameters for 33 subjects, except for sex and ethnicity, where the *n* (percentage) is shown.

Results

Thirty-three healthy normal volunteers completed the study without dose modification or study withdrawal (Table 1). Total number of subjects who were randomized is 33. Seventeen subjects were assigned to sequence 1 (OxDC to placebo), and 16 subjects were assigned to sequence 2 (placebo to OxDC) (Figure 1).

The mean 24-hour Uox (mean ± SEM) increased from 19.81±0.92 mg/24 h at the time of screening (days −28 to −3) to 44.7±1.25 mg/24 h during the high-oxalate diet equilibration period (day −1) before study product administration in treatment period 1 ($P<0.001$). The mean 24-hour Uox (mean ± SEM) was 40.29±1.34 mg/24 h during the crossover washout period (days 3 and 4) when the subjects were still on the high-oxalate diet before beginning treatment period 2. Mean Uox on the placebo was 39.59±1.20 mg/24 h (days 1 and 2 for sequence 2 and days 5 and 6 for sequence 1). Mean Uox on OxDC was 30.14±0.93 mg/24 h ($P<0.001$; days 1 and 2 for sequence 1 and days 5 and 6 for sequence 2) (Figure 2).

The baseline corrected within-subject mean reduction in 24-hour Uox excretion was 12.46 mg or 29.25% ($P<0.001$). The 24-hour Uox excretion in the placebo group was affected (40 mg/24 h or 6.9%) compared with average baseline levels established after consuming the controlled high-oxalate diet ($P=0.004$). OxDC produced a 23.87% reduction ($P<0.001$) in 24-hour Uox excretion compared with placebo. Approximately 65% of subjects (21 of 33) had a 20% or higher reduction in Uox, and there was >5% reduction in 94% of subjects (31 of 33) after administration of OxDC. When comparing placebo with OxDC, 11 subjects had ≥30% reduction, 21 subjects had ≥20% reduction, and 28 subjects had ≥10% reduction in Uox (Table 2). After correcting for day-to-day variation in Uox excretion (comparing baseline periods), 58% of subjects (18 of 31) had a reduction over 15%, and 55% (17 of 31) had a reduction over 20%. Other urinary parameters did not change significantly between OxDC and placebo treatment (Figure 3).

All subjects were satisfied with the number of meals consumed and dose of study product. One urine sample was disqualified because the individual did not comply with study diet and ate <90% of the oxalate in one meal. Two urine samples were disqualified from the study due to a deviation, which had no significant effect in our results.

No SAEs were reported during the study. Five TEAEs during OxDC treatment were classified as mild, and one (vomiting) was classified as moderate in severity. Three subjects reported four TEAEs during placebo treatment (one report each of stiffness of back, stiffness of neck, menstrual cramps, and heartburn). All TEAEs were resolved by the end of study. No clinically significant abnormal clinical test results were reported during the study, and all subjects completed the study.

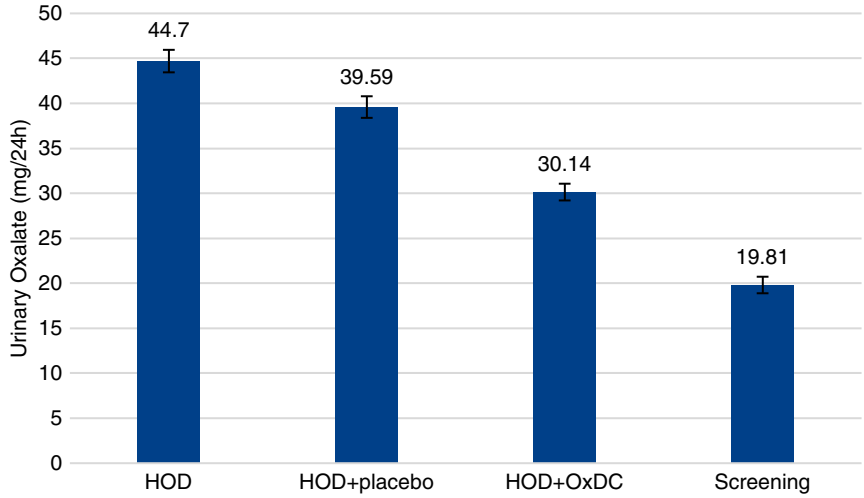

Figure 2. | **Urinary oxalate excretion in different study periods.** Urinary oxalate excretion during high-oxalate diet (HOD) equilibration, HOD and placebo, and HOD and oxalate decarboxylase (OxDC) compared with screening excretion.

Discussion

Hyperoxaluria can lead to recurrent kidney stones (5). Oxalate, amply found in plant sources, is the ionic form of oxalic acid, which is an end product of human metabolism (5,6). Oxalate is absorbed throughout the gastrointestinal tract, beginning in the stomach (7), and it is normally excreted through the kidneys because it does not seem to be needed for any process in the human body (6). Although the oxalate concentration in the urine is simply one-tenth that of calcium (8), calcium oxalate is close to its supersaturation limit; thus, a small increase in oxalate concentration can increase the risk of crystal precipitation (9). That oxalate is a continuous variable when considering stone risk has also been demonstrated in large epidemiologic cohort studies (10). Calcium oxalate supersaturation in the urine and the lack of adequate hydration can lead to calcium oxalate kidney stone formation, although the process is complex and may involve other factors (11).

Few promising pharmaceutical therapies have emerged over the last decade for the prevention and management of kidney stones (1,3). Dietary modifications, including a low-oxalate and low-sodium diet and normal intakes of calcium (1000–1200 mg/d) coupled with adequate fluid intake, are recommended and considered successful for the prevention of hyperoxaluria and kidney stone recurrence (1,3). However, compliance with a low-oxalate, low-sodium, and normal calcium-level diet is difficult to maintain because oxalate is present in many different foods, and its level in each food varies considerably (12). In the typical Western diet, the intake of oxalate can range from an average of 80 to 120 mg/d but can be as high as 350 mg/d (12–14). Calcium intake continues to be low in many individuals. According to the Women's Health Initiative Observational Study that evaluated dietary factors and incidence of kidney stone formation, approximately 80% of the women in the study consumed less than the recommended daily dietary calcium intake of 1000–1200 mg/d, which may have predisposed them to kidney stones (15).

In 2007, a randomized, controlled trial utilizing Oxadrop, a mix of four lactic acid bacterium species, did not reduce Uox excretion in patients with idiopathic hyperoxaluria (16). In 2010, another study was performed comparing diet and two probiotic combinations (Oxadrop and AKSB). Dietary oxalate restriction reduced urinary excretion and calcium oxalate supersaturation, but the probiotics did not influence Uox levels in the subjects (17). In 2013, the role of *Oxalobacter formigenes*, a bacterium that degrades oxalate in the intestinal tract, was evaluated in terms of colonization in calcium oxalate stone disease; its presence was associated with a reduced risk of calcium oxalate stone formation, and its absence was associated with an increase in Uox excretion and risk of stone formation (18).

A double-blind, placebo-controlled, randomized, crossover study with ALLN-177, an orally administered oxalate-degrading enzyme, evaluated its effect on reducing the absorption of oxalate and excretion in the urine. ALLN-177 is an encapsulated recombinant OxDC from *Bacillus subtilis* expressed in *E. coli* (1500 U per capsule). When compared with placebo, ALLN-177 treatment reduced Uox by 11.6±2.7 mg/24 h ($P<0.001$; least squares mean ± SD) (19). Treatment with ALLN-177 was effective in 63% (19 of 30) of subjects, and treatment with OxDC herein was effective in 94% (31 of 33) of subjects. In the study by Langman *et al.* (19), the men quota was higher (27:6 men: women versus 17:16 men:women herein); however, age and body mass index were comparable (average age 39.7 [10.7] versus average age 36.2 [12.27] herein). There were notable differences in baseline Uox levels between the study evaluating ALLN-177 and our study described herein; baseline Uox was 80.8±24.1 mg/24 h (mean ± SD; minimum: 46.3, maximum: 144.5) versus 44.7±7.2 mg/24 h (mean ± SD; minimum: 31.7, maximum: 58.1) described herein. It is further noted that the dosage form is different (capsule versus sachet). In our study, the dosage form and way of administration would ensure immediate dispersion in the stomach compared with a capsule dosage form, which can reasonably be expected to have longer relative dispersion time. Other differences between the study by Langman *et al.* (19) and our study were the levels of oxalate and calcium ingested, the study period lengths, and screening urine

Table 2. Urinary oxalate on high-oxalate diet (day −1) and average change between placebo and oxalate decarboxylase treatment periods

Subject Identification, n=33	Urinary Oxalate High-Oxalate Diet,[a] 24-h Urine (mg/24 h)	Urinary Oxalate (Oxalate Decarboxylase Minus Placebo),[b] 24-h Urine (mg/24 h)	Reduction,[c] %
9008	52.8	−19.75	41.19
9030	43.1	−16.75	38.42
9032	45.8	−6.65[d]	21.28[d]
9009	51.9	−16.7	36.15
9007	37	−15.85	35.3
9012	46.7	−13.65	34.08
9006	51.9	−14.55	34.07
9019	40.5	−11.9	33.38
9017	48.4	−15.85	32.75
9018	58.1	−16.75	31.97
9028	58.1	−15.9	31.12
9011	42.2	−10.55	30.36
9015	41.4	−10.5	29.13
9004	51.9	−10.95	27.97
9024	40.5	−10.5	27.78
9029	50.2	−13.2	26.77
9001	44	−9.65	25.23
9014	37	−7.5	24.71
9020	52.8	−10.15	23.52
9003	42.2	−7.45	21.16
9021	40.5	−8.35	20.42
9033	37.8	−8.75	19.13
9005	44	−7	18.09
9016	29.9	−5.75	17.64
9023	55.5	−5.25	16.13
9027	42.2	−6.15	16.06
9026	47.5	−6.6	15.62
9010	35.2	−3.05	13.59
9031	37	−2.65[d]	8.14[d]
9013	42.2	−3.45	9.13
9002	31.7	−2.25	6.47
9022	44	0	0
9025	50.2	2.15	−6.78

[a]Mean of urinary oxalate during equilibration period on high-oxalate diet (day −1) immediately preceding the administration of the study drug.
[b]The values represent the difference of urinary oxalate (milligrams per 24 hours) between placebo and oxalate decarboxylase treatment periods.
[c]The values represents the percent reduction in urinary oxalate calculated as [(mean placebo – mean oxalate decarboxylase)/mean placebo] ×100.
[d]Because of an error in sample pooling upon urine collection, the day 1 collections of Subject 9031 and Subject 9032 were excluded from calculation.

levels (Table 3). The dose of enzyme was substantially higher in the Langman *et al.* (19) study than in this study (7500 U of ALLN-177 was given per meal for a total of 22,500 U/d versus 1000 U of OxDC per meal for a total of 3000 U/d). The fact that OxDC described herein produced statistically significant results at a lower dose and at lower average and maximum Uox levels could mean that these products differ in certain enzymatic properties, such as the catalytic efficiency or Michaelis-Menten constant (K_m). An enzyme's K_m is an inherent characteristic that is defined as the substrate concentration at which the enzyme demonstrates 50% of its maximum rate of reaction. Thus, we hypothesize that the OxDC enzyme considered here has a higher catalytic efficiency or a lower K_m and therefore, demonstrates 50% of its maximum rate of reaction at a lower oxalate concentration. As a result, the OxDC as described herein more effectively degrades oxalate in a low-substrate environment.

There were some limitations of this study. During screening, urine samples were collected by participants at home and not in a controlled environment. This could explain why the screening urine was relatively low in Uox levels: 19.82±5.3 mg/24 h (normal range: 9.7–40.5 mg/24 h). Urine collected during screening was only used for reducing the risk of enrolling subjects with secondary hyperoxaluria. Additionally, this study was performed on healthy normal volunteers in a research center where most factors were controlled. Results could differ if the study included subjects with kidney stones and/or subjects who did not consume a diet so high in oxalate or low in calcium (herein used to ensure maximum exposure). This study had a 2-day dosing period, but the intended use of this enzyme is long term; thus, this is a limitation of study design. An effect from an altered microbiome upon introduction of a new diet cannot be excluded in particular because these are subjects with presumed healthy gut biome; for example, the small but

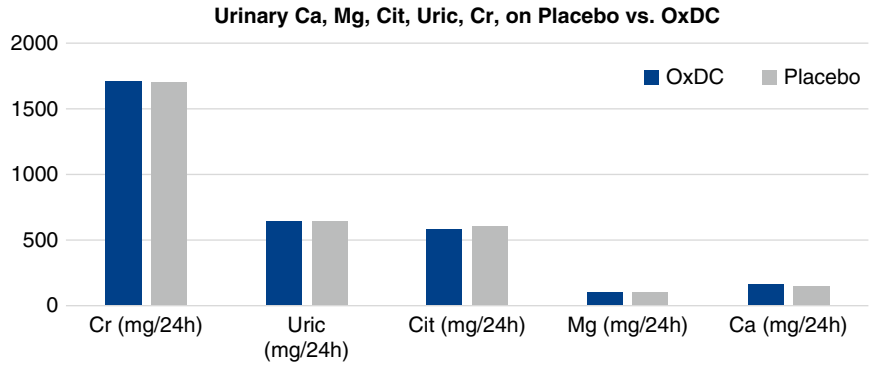

Figure 3. | **No significant change in other urinary parameters measured.** Excretion of additional urinary parameters measured (calcium [Ca], magnesium [Mg], citrate [Cit], uric acid [Uric], and creatinine [Cr]) during placebo dose versus OxDC dose.

notable reduction in baseline level could be attributed to increased activity of oxalate-degrading microbes in the gut. Another limitation is that the level of colonization with *O. formigenes* in our study participants was not controlled, and its absence may be associated with an increase in Uox excretion (18).

Treatment with OxDC at doses of 1000 U (micromoles per minute per milligram) with meals three times per day resulted in a significant reduction in Uox excretion levels. After dosing with OxDC, the baseline corrected within-subject mean reduction in 24-hour Uox excretion was 12.46 mg or 29.25% ($P<0.001$) from an average baseline of 42.5 mg Uox excretion; thus, the OxDC treatment effectively degraded oxalate in a low-substrate environment. OxDC treatment demonstrated a positive effect (>5% reduction) in 31 of 33 subjects (94%), and when correcting for day-to-day variation, a clinically significant effect (>20% reduction) was observed in 17 of 31 (55%) subjects. Compared with placebo, OxDC produced a 23.87% reduction ($P<0.001$) in 24-hour oxalate excretion. OxDC was well

Table 3. Comparison of trials evaluating oxalate decarboxylase enzymes in healthy volunteers

Langman *et al.* (19)	This Study
Study product	
Recombinant OxDC originating from *Bacillus subtilis*	Recombinant OxDC originating from *Synechoccus elongates*
Capsule format	Sachet format
1500 U per capsule	1000 U per sachet
Five capsules per dose	One sachet per dose
Participants	
33 healthy volunteer enrolled	33 healthy volunteers enrolled
30 healthy volunteers completed study	33 healthy volunteers completed study
27:6 men:women	17:16 men:women
Average age 39.7 (10.7)	Average age 36.2 (12.27)
Average BMI: 24.9	Average BMI: 24.9
Baseline Uox: 80.8 mg/24 h	Baseline Uox: 44.7 mg/24 h
Screen Uox: 27.2 mg/24 h	Screen Uox: 19.8 mg/24 h
Study design	
Double blind	Double blind
Randomized	Randomized
Placebo controlled	Placebo controlled
Crossover	Crossover
Inpatient study	Inpatient study
Treatment period: 7 d	Treatment period: 2 d
Washout period: 7 d	Washout period: 2 d
Follow-up visit: 7 d	Follow-up visit: none
Diet oxalate: 1000 mg/d	Diet oxalate: 750 mg/d
Diet calcium: 400 mg/d	Diet calcium: 550 mg/d
Outcome measured over last 4 d of 7-d treatment period	Outcome measured over the 2 d of treatment period
Interim analysis	No interim analysis
22,500 U/d	3000 U/d

OxDC, oxalate decarboxylase; BMI, body mass index; Uox, mean urinary oxalate.

tolerated by all study participant. OxDC is also generally recognized as safe. There were no SAEs reported during the study. This study provides clinically meaningful data that can help further our understanding of ways to reduce urinary oxalate excretion in patients with calcium oxalate stones and secondary hyperoxaluria.

Disclosures

S. Buzzerd, I. Klimberg, H. Liu, A. Ryan, and G. Stevens report personal fees from Oxidien Pharmaceuticals for consulting work. S. Buzzerd reports personal fees from Dynamic Clinical Research, outside the submitted work. H. Liu reports grants from QPS Qualitix Clinical Reserch Co., Ltd. during the conduct of the study. All remaining authors have nothing to disclose.

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Author Contributions

V. Bird and A. Ryan conceptualized the study; V. Bird, S. Buzzerd, I. Klimberg, H. Liu, E. Quintero, A. Ryan, and G. Stevens were responsible for investigation; H. Liu and G. Stevens were responsible for data curation and formal analysis; S. Buzzerd and I. Klimberg provided supervision; E. Quintero wrote the original draft; and V. Bird, S. Buzzerd, I. Klimberg, H. Liu, E. Quintero, A. Ryan, and G. Stevens reviewed and edited the manuscript.

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