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A Prospective, Double-Blind, Randomized, Placebo-Controlled, Cross-Over 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, hyper absorption of oxalate, or increased endogenous production of oxalate. The disorder spectrum extends from recurrent kidney stones to end stage renal disease. 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, cross-over clinical trial 33 healthy volunteers were randomized into 2 cross-over 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 ~1,000 units (umol/min/mg) of OxDC or placebo with meals 3 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 vs. high oxalate baseline preceding treatment) was 12.5 mg or 29% ($p < 0.0001$). OxDC treatment was effective (> 5% reduction) in 31/33 subjects (94%). Compared with placebo, OxDC produced a 24% reduction ($p < 0.0001$) 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.

Conclusion 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.

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 may be associated with excessive oxalate intake in the diet, 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, bariatric surgery) or inflammatory bowel disease leading to increased oxalate absorption. Primary hyperoxaluria (Types I-III) 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 calcium oxalate stone patients with 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 the acidic pH of the stomach. The present study considers the extent to which OxDC vs. 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, USA) approval was obtained prior to study initiation (IRB Approval Letter 07/11/2018) and registered in

clinicaltrials.gov (NCT03661216 Date of Registration 09/07/2018). All subjects provided written informed consent with guarantee of confidentiality.

This was a prospective, double-blind, randomized, placebo-controlled, cross-over study conducted in a confined clinical setting Bio-Kinetic Clinical Applications, LLC , Springfield, MO) for 9 days, under Good Clinical Practices (GCP), in accordance with FDA Guidelines and the Declaration of Helsinki. Study recruitment initiated July 2018 and last subject last visit was conducted 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/day) and low calcium intake (500-550 mg/day). Subjects started the controlled diet on Day -2 (equilibrium period). Block randomization was implemented with blinded randomization cards assigning into two different cross-over 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 two days (Day 1 and Day 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 1,000 units (umol/min/mg) of OxDC or placebo with meals 3 times per day during the 4 treatment days. Study product was suspended in approximately 8 oz 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 1000U/sachet. One unit of activity is the conversion of 1umol of oxalate to formate per minute and mg of enzyme at 37ºC and pH 3.

Study Population

All 33 subjects had the following entry criteria: age of 18-55 years, BMI of 18.5 – 29.9 kg/m^2, eGFR of ≥ 60 mL/min/1.73 m^2, urinary oxalate ≤ 40.5 mg/24 hours, and urinary uric acid < 750 mg/24 hours. Male and non-pregnant females who were non-smokers for 3 months at time of screening and throughout 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. Female subjects agreed to use an acceptable form of birth control from screening through the duration of the study.

Urine Analysis

Mayo Clinic laboratory in Rochester, MN 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 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, MO 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 second 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 Analysis

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

Statistical Analysis

The sample size was calculated to detect a mean urinary oxalate (Uox) difference of 5 mg/24 hours when comparing OxDC or placebo. The estimated sample size was 14 subjects to achieve a power of 90% and significance level of 0.05 (2-sided) using the paired t-test.

The endpoints 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 vs. 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 (SAS®) (release 9.3 or higher).

Baseline is defined as the Uox (mg/24h) for the high oxalate diet period preceding treatment (Day -1 and Day 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.

Results

Thirty-three healthy normal volunteers completed the study without dose modification or study withdrawal (see 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 ± SE) increased from 19.81 ± 0.92 mg/24h at the time of screening (Day -28 to -3) to 44.7 ± 1.25 mg/24h during the high oxalate diet equilibration period (Day-1) before study product administration in treatment period 1 ($p < 0.0001$). The mean 24-hour Uox (mean ± SE) was 40.29 ± 1.34 mg/24h during the cross-over washout period (Days 3 to 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/24h (Day 1 to 2 for sequence 2 and Day 5 to 6 for sequence 1). Mean Uox on OxDC was 30.14 ± 0.93 mg/24h ($p <0.0001$) (Day 1 to 2 for sequence 1 and Day 5 to 6 for sequence 2) (see Figure 2).

The baseline corrected within-subject mean reduction in 24-hour Uox excretion was 12.46 mg or 29.25% ($p < 0.0001$). The 24-hour Uox excretion in the placebo group was affected (40 mg/24hr or 6.9%) compared with average baseline levels established after consuming the controlled high oxalate diet (HOD) ($p = 0.0042$). OxDC produced a 23.87% reduction ($p < 0.0001$) in 24-hour Uox excretion compared to placebo. Approximately 65% of subjects (21/33) had a 20%

or higher reduction in Uox and there was > 5% reduction in 94% of subjects (31/33) 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 (18/31) had a reduction over 15% and 55% (17/31) 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 less than 90% of the oxalate in one meal. Two urine samples were disqualified from the study due to a deviation, which had no significant impact in our results.

No SAEs were reported during the study. Five TEAEs during OxDC treatment were classified as mild and 1 (vomiting) as moderate in severity. Three subjects reported 4 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 study.

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 (GI), beginning in the stomach (7), and is normally excreted through the kidneys since it does not seem to be needed for any process in the human body (6). Though 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 have also been demonstrated in large epidemiological 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 diet, low sodium diet, and normal intakes of calcium (1,000-1,200 mg/day) 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 80-120 mg/day but can be as high as 350 mg/day (12-14). Calcium intake continues to be low in many individuals. According to the Women's Health Initiative Observational Study, evaluating dietary factors and incidence of kidney stone formation, approximately 80% of the women in the study consumed less than recommended daily dietary calcium intake of 1,000 to 1,200 mg per day, which may have predisposed them to kidney stones (15).

In 2007, a randomized, controlled trial utilizing Oxadrop, a mix of 4 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, 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 *Escherichia coli* (1,500 units/capsule). When compared with placebo, ALLN-177 treatment reduced Uox by 11.6 ± 2.7 mg/24h, p = 0.0002 (least squares mean ± SD) (19). Treatment with ALLN-177 was effective in 63% (19/30) of subjects, and treatment with OxDC herein was effective in 94% (31/33) of subjects. In Langman's study, the male participant quota was higher (27:6 men:women vs. 17:16 men:women herein); however, age and BMI were comparable (avg. age 39.7 (10.7) vs. avg. 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 hour (mean ± SD) (min: 46.3 max: 144.5) vs. 44.7 ± 7.2 mg/24 hour (mean ± SD) (min: 31.7 max: 58.1) described herein. It's further noted that the dosage form is different (capsule vs. sachet). In our study, the dosage form and way of administration would ensure immediate dispersion in the stomach as compared to a capsule dosage form which can reasonably be expected to have longer relative dispersion time. Other differences between Langman's study and ours were the level of oxalate and calcium ingested, the study period lengths and screening urine levels, see Table 3. The dose of enzyme was substantially higher in the Langman study than in the present study (7,500 units of ALLN-177 were given per meal for a total of 22,500 units per day vs. 1,000 units of OxDC per meal, for a total of 3,000 units per day). The fact that OxDC described herein produced statistically significant results at a lower dose and at

lower average and maximum Uox level could mean that these products differ in certain enzymatic properties, such as the catalytic efficiency or Km. An enzyme's Km 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 Km 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/24h (normal range: 9.7-40.5 mg/24h). 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 impact from an altered microbiome upon introduction of a new diet cannot be excluded in particular since these are subjects with presumed healthy gut biome; for example, the small but 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 *Oxalobacter formigenes* in our study participants was not controlled and its absence may be associated with an increase in Uox excretion (18).

Conclusions

Treatment with OxDC at doses of 1,000 units (umol/min/mg) with meals 3 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 % ($p < 0.0001$) 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 31/33 subjects (94%) and when correcting for day-to-day variation a clinically significant effect (>20% reduction) was observed in 17/31 (55%) subjects. Compared with placebo, OxDC produced a 23.87% reduction ($p < 0.0001$) in 24-hour oxalate excretion. OxDC was well 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 calcium oxalate stone patients with secondary hyperoxaluria.

Disclosures

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

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Table 1. Baseline Subject Characteristics

Parameters	Value
Age, years	36.2 ± 12.27
Height (cm)	169.3 ± 8.30
Weight (kg)	71.18 ± 11.937
Gender, n (%)	
Male	17 (51.52)
Female	16 (48.48)
Race, n (%)	
White/Caucasian	29 (87.88)
Black/African American	3 (9.09)
Other	1 (3.03)
eGFR, ml/min/1.73 m^2	96.8 ± 18.07

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

Table 2. Urinary oxalate on high oxalate diet (Day-1) and average change between placebo and OxDC treatment periods

Subject ID	Urinary Oxalate high oxalate diet[1]	Urinary oxalate (OxDC minus Placebo)[2]	
n=33	24h urine (mg/24h)	24h urine (mg/24h)	% Reduction
9008	52.8	-19.75	41.19
9030	43.1	-16.75	38.42
9032	45.8	-6.65*	21.28*
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*	8.14*
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

[1]Mean of urinary oxalate during equilibration period on high oxalate diet (Day -1) immediately preceding the administration of the study drug.
[2]The values in the first column represent the difference of urinary oxalate (mg/24h) between placebo and OxDC treatment periods. The second column of values represents the percent (%) reduction in urinary oxalate calculated as [(mean placebo – mean OxDC / mean placebo] x100.
* Due to an error in sample pooling upon urine collection the Day 1 collection of Subject 9031 and Subject 9032 were excluded from calculation.

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

	Langman *et. al.* 2016	Quintero *et. al.* 2019
Study Product	Recombinant OxDC originating from *B. subtilis*	Recombinant OxDC originating from *S. elongates*
	Capsule format	Sachet format
	1,500 units per capsule	1,000 units per sachet
	5 capsules per dose	1 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
	Avg. age 39.7 (10.7)	Avg. age 36.2 (12.27)
	Avg. BMI: 24.9	Avg. BMI: 24.9
	Baseline Uox: 80.8 mg/24h	Baseline Uox: 44.7 mg/24h
	Screen Uox: 27.2 mg/24h	Screen Uox: 19.8 mg/24h
Study Design	Double-blind	Double-blind
	Randomized	Randomized
	Placebo-controlled	Placebo-controlled
	Cross-over	Cross-over
	Inpatient study	Inpatient study
	Treatmetn period: 7 days	Treatment period: 2 days
	Wash-out period: 7 days	Wash-out period: 2 days
	Follow-up visit: 7 days	Follow-up visit: none
	Diet oxalate: 1000 mg/day	Diet oxalate: 750 mg/day
	Diet calcium: 400 mg/day	Diet calcium: 550 mg/day
	Outcome measured over last 4 days of 7 day treatment period	Outcome measured over the 2 days of treatment period
	Interim analysis	No interim analysis
	22,500 units/day	3,000 units/day

Figure 1.

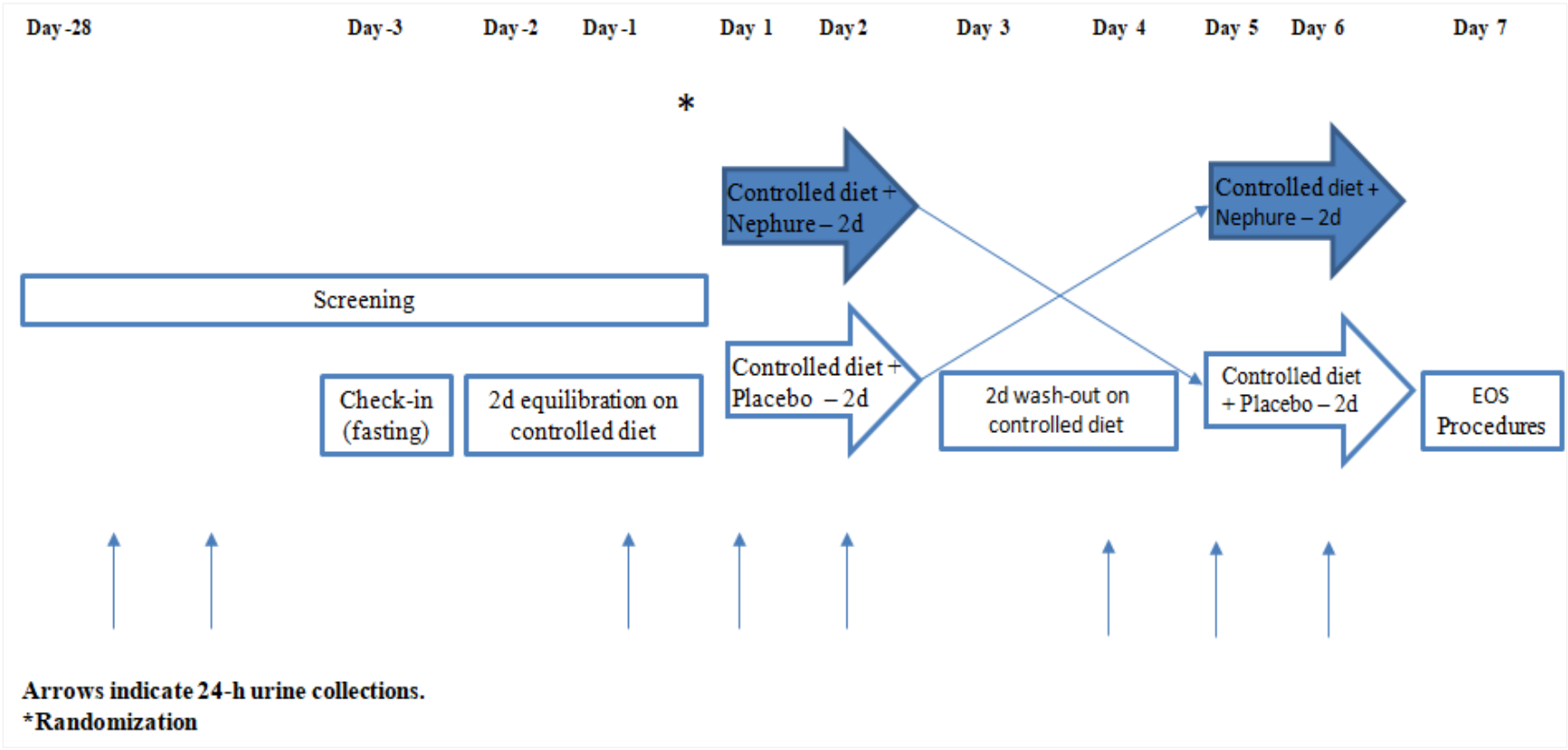

Figure 1. Study Design. The study included a screening period that began 28 days before randomization of subjects occurred, in which two 24-hour urine collections were performed. Following screening, subjects conducted six (6) additional 24-hour urine collections (with Collection Start Time on Day -1, 1, 2, and Day 4, 5, 6). The vertical arrows signify when the urine collections occurred. Sequence 1 of the study includes Day 1 and Day 2. Sequence 2 consists of Day 5 and Day 6.

Figure 2.

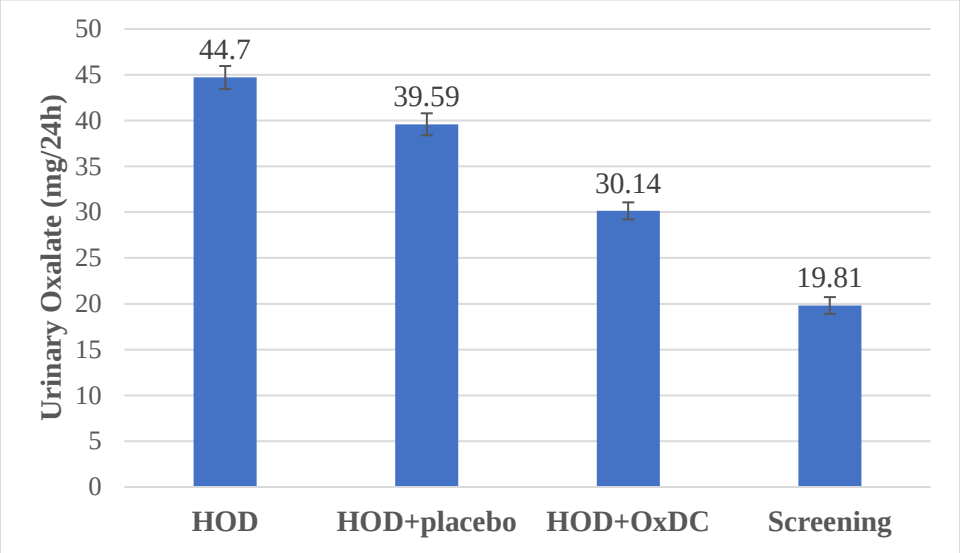

Figure 2. Urinary oxalate excretion during high-oxalate diet equilibration (HOD), high-oxalate diet and placebo, high-oxalate diet and OxDC, as compared to screening excretion.

Figure 3.

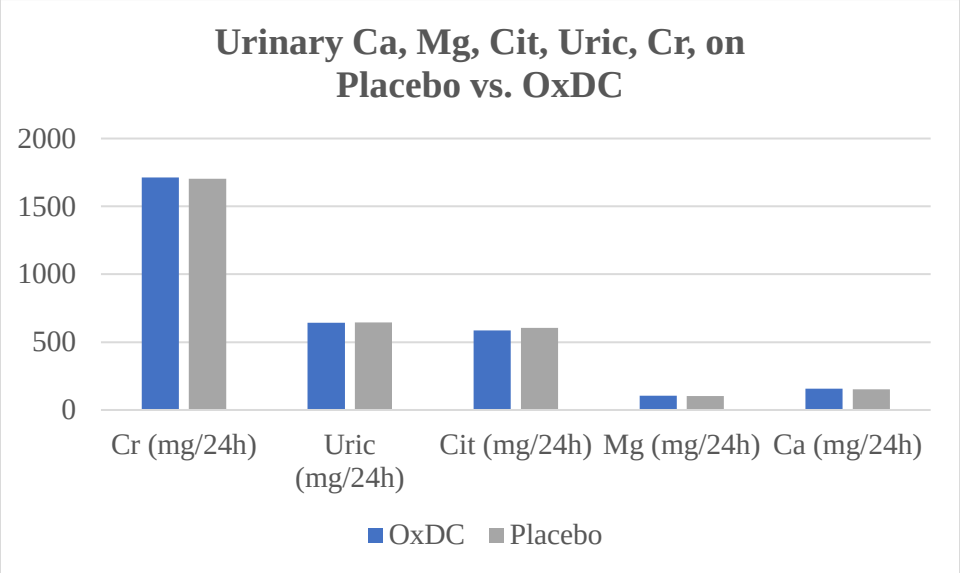

Figure 3. Excretion of additional urinary parameters measured; calcium (Ca), magnesium (Mg), citrate (Citrate), uric acid (Uric), creatinine (Cr) during placebo dose vs. OxDC dose.