A Randomized, Controlled Trial of Lactic Acid Bacteria for Idiopathic Hyperoxaluria

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Background: Urinary oxalate excretion is an important contributor to calcium oxalate stone formation. Methods of reducing oxalate excretion are not wholly satisfactory, and no controlled trials using them have been performed to prevent stone recurrence. Some lactic acid bacteria can degrade oxalate *in vitro*. This study sought to reduce urinary oxalate excretion in calcium stone formers with idiopathic hyperoxaluria.

Design, setting, participants, and measurements: A randomized, double-blind, placebo-controlled trial was performed of Oxadrop, a mix of four lactic acid bacterium species. This preparation previously reduced oxalate excretion in stone formers with idiopathic and enteric hyperoxaluria. Patients were selected from two stone prevention clinics. Twenty people with calcium stones and idiopathic hyperoxaluria (>40 mg/d) were enrolled and randomly assigned 1:1 in placebo and active preparation arms. Both groups took 3.6 g of granulate each day: Either placebo or the experimental preparation. Participants performed two consecutive 24-h urine collections at baseline, at 28 d of therapy, and at 56 d, after being off the preparation for 4 wk. Diet was replicated at each point.

Results: There was no effect of the study preparation: Mean 24-h urinary oxalate excretion in placebo-treated patients was 73.9 mg at baseline and 72.7 mg after treatment, whereas the Oxadrop-treated patients had 59.1 mg at baseline and 55.4 mg after treatment. The preparation was well tolerated; three participants on active treatment experienced mild constipation.

Conclusions: In this randomized, placebo-controlled trial, Oxadrop did not reduce urinary oxalate excretion in participants with idiopathic hyperoxaluria.

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Calcium oxalate is the most common crystal constituent of kidney stones. Hyperoxaluria, usually defined as urinary excretion of >40 mg/d, is an important risk factor for stones and is present in approximately 20 to 40% of stone formers (1,2). The sources of urinary oxalate in adults who do not have primary hyperoxaluria are increased dietary oxalate intake, increased enteral oxalate absorption, or increased endogenous production by metabolism. Specific treatments for hyperoxaluria are limited; these treatments are mostly based on dietary restrictions and may not be applicable to people who cannot identify causative dietary constituents in their own diet. Despite good evidence of the role of oxaluria in stone formation, no randomized, controlled trials have shown that lowering oxalate excretion is associated with prevention of stone recurrence.

Recent studies have shown that components of the endogenous intestinal microbiota can utilize oxalate, potentially limiting its absorption from the intestinal lumen. Campieri *et al.* (3) demonstrated that a preparation of lactic acid bacteria reduced urinary oxalate excretion by 40% in an uncontrolled study of calcium stone–forming participants with idiopathic hyperoxaluria. The same preparation was associated with a 24% reduction in urinary oxalate in participants with enteric hyperoxaluria (4). We sought to confirm this finding using the same preparation in a randomized, double-blind, placebo-controlled trial in participants with idiopathic hyperoxaluria.

Materials and Methods

Study Design

This was a randomized, double-blind, placebo-controlled trial of a preparation of lactic acid bacteria. Allocation was concealed. The sample size of 20, with 10 patients in each arm, was calculated to have a power to detect a 25% reduction in urinary oxalate excretion with 80% sensitivity.

Study Patients

Study participants who were capable of giving informed consent were recruited for the study from the Kidney Stone Prevention Programs at the New York Harbor Department of Veterans Affairs Medical Center and the St. Vincent's Catholic Medical Center, both in New York, NY. The study was approved by both institutional review boards. Eligible participants included men and women with a history of at least one calcium oxalate kidney stone and hyperoxaluria ≥40 mg/d. When a stone analysis was available, the stone had to be at least 50% calcium oxalate. Participants who had not had a stone analysis were eligible when their stone was known to be radio-opaque, consistent with cal-

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cium composition, and hyperoxaluria was present. Twelve of 20 participants had a stone analysis; eight had radio-opaque stones and no stone analysis. Hyperoxaluria had to have been present on all 24-h urine collections performed before the study in the course of their usual evaluation for prescription of a preventive strategy (mean number of collections 3.6; range 1 to 9). These prestudy collections were performed either on the patients' *ad libitum* diets or on diets that were prescribed for stone prevention; participants with sporadic hyperoxaluria were thereby excluded. Patients with urinary abnormalities in addition to hyperoxaluria, including low urine volume, hypercalciuria, hypocitraturia, and hyperuricosuria, were not excluded. Patients were included when they were taking drugs for the prevention of stone disease, including pyridoxine, thiazides (chlorthalidone, hydrochlorothiazide, and indapamide), citrate supplements, and allopurinol, as long as there had been no changes in these prescriptions for at least the 3 previous months.

Patients were excluded from participation when they had a history of inflammatory bowel disease, ileal or colonic resection, or bariatric surgery; when they had cancer that was not in remission or were receiving chemotherapy; when they were taking steroids or other immunosuppressants; or when they had infection with HIV. We excluded patients with primary hyperoxaluria or those who were treated with Ox-Absorb, cholestyramine, or calcium supplements. Also excluded were patients who completed a course of oral or parenteral antibiotics <2 wk before initiation of the study. We planned to disqualify participants who required a course of antibiotics during the study, but this event did not occur.

Study Preparation

The study preparation is a proprietary freeze-dried formulation called Oxadrop (VSL Pharmaceutical, Gaithersburg, MD). Each gram of the study preparation contains 3.6×10^{11} bacteria. The preparation is assayed at the time of packaging for colony-forming units so that the number of organisms contained represents only the number of viable cells. Preparations with colony-forming units less than specified are rejected. The preparation has been shown to be stable with respect to viability when kept refrigerated up to 2 yr. The preparation was shipped on dry ice, and participants were instructed to keep it refrigerated. According to the manufacturer, oxalate degradation is proportional to the number of viable cells; at 2 yr, oxalate degradation of the preparation had not changed. This study was completed in <2 yr, so stability of the preparation beyond the point at which testing had established viability was not necessary.

The organisms included *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Streptococcus thermophilus*, and *Bifidobacteria infantis*. Each species is grown separately before mixing. These four species were mixed in a 1:1:4:4 ratio, respectively, and prepared as a granulate. Each packet contained 3 g of material. The placebo was a very similar-appearing preparation of corn starch in identical packets. All packets of the active preparation used in this study were derived from a single batch and were made from the same batch as that used in a previously published article of a study of participants with enteric hyperoxaluria (4). Because the preparation has not yet been made commercially available, only two batches have been made. Although produced by the same manufacturer, the preparation that we used differed from that used in an earlier study in that the current product did not contain a strain of *Lactobacillus plantarum*, which had no significant ability to degrade oxalate (3). As further quality control, each batch is assayed and confirmed to be negative for contamination with other species such as enterococcal species, *Listeria*, coliforms, *Escherichia coli*, *Salmonella* species, and *Staphylococcus aureus*.

Study Procedure

Patients collected two consecutive 24-h urine samples on three occasions: (*1*) baseline, (*2*) days 27 and 28 at the end of 4 wk of therapy, and (*3*) days 55 and 56 after being off therapy for 4 wk. After signing an informed consent, the participants undertook the collection of the baseline set of two consecutive 24-h urine samples. During the collection of the baseline urine samples, patients were asked to document their diet in a food diary. Participants ate their *ad libitum* diets. In the course of their prestudy medical care, all had been advised regarding restriction of oxalate ingestion and, in appropriate cases, restriction of salt and animal protein intake as well. Diet diaries were used by study participants to replicate dietary intake but not collected by the investigators. When this first set of urine samples confirmed hyperoxaluria, the patients were randomly assigned to one of two groups: Active preparation or placebo. Patients who did not have hyperoxaluria (>40 mg/d) at baseline, despite having had it previously, were excluded from the protocol.

Participants then took one packet of either the study preparation or the placebo once each day for a period of 28 d. The preparation was to be mixed with any cold beverage except milk. Patients were instructed to take the preparation 1 to 2 h after dinner or the major meal of the day. On days 27 and 28 of taking the assigned preparation, the patients again performed two 24-h urine collections. During these 2 d, they attempted to replicate the diet, recorded on the food diaries, that they had ingested during collection of the baseline samples. The patients, then off therapy, waited another month and on days 55 and 56 again replicated the diet while performing two more 24-h urine collections. At the end of the study, a brief questionnaire was administered by mail to determine how participants tolerated the preparation and whether they were able to identify their assignment.

Urine Chemistries

Urine collections were maintained at room temperature. An antimicrobial preservative and a volume marker were added to each urine container. At the end of the 24-h collection, the urine collection container was shaken and a 50-ml aliquot was obtained and sent by overnight mail to Litholink Corp. (Chicago, IL) for analysis.

Twenty-four-hour urinary concentrations of oxalate, calcium, and other determinants of supersaturation were measured using standard laboratory assay techniques on a Beckman-Synchron CX5 (Beckman Instruments, Brea, CA) (5). Oxalate was measured by enzyme assay using oxalate oxidase (Sigma Chemical Co., St. Louis, MO). Supersaturation was calculated using the EQUIL2 program (6).

Statistical Analyses

Group means at each time period were compared by two-tailed paired *t* test using SPSS (SPSS, Chicago, IL). When results were not normally distributed, the Kruskal-Wallis test was performed. Results were expressed as means ± SD. $P < 0.05$ was accepted as significant.

Role of the Sponsor

The study was initiated by the investigators and funded by VSL Pharmaceuticals. Every aspect of the study was designed by the investigators except for the study preparation. The sponsor supplied both the study preparation and an identically packaged placebo. The sponsor agreed in advance not to have a role in decisions regarding submission of a manuscript and did not offer any revisions of the manuscript when it was completed.

Results

In total, 38 patients agreed to participate and signed consent. Five dropped out without completing the study before ran-

domization for personal reasons. Thirteen patients were excluded because their baseline urinary oxalate excretion was <40 mg/d. Twenty patients, 10 in each group, completed the study. The mean age of the participants who completed the study was 60.0 ± 13.1 yr for the placebo and 57.3 ± 8.2 yr for the active preparation. The participants included 19 men and one woman.

There was no effect of the active preparation on urinary oxalate excretion (Figure 1). Because the results were not normally distributed, we analyzed the results by Kruskal-Wallis test and demonstrated that there was no statistically significant effect of the preparation. The median 24-h urinary oxalate excretion in placebo-treated patients was 57.6 mg (interquartile range [IQR] 45.4 to 104.0 mg) at baseline, 62.0 mg (IQR 49.7 to 79.8 mg) with treatment, and 56.8 mg (IQR 46.2 to 76.1 mg) 1 mo after treatment. The Oxadrop-treated patients had 56.2 mg (IQR 48.9 to 65.2 mg) at baseline, 52.6 mg (IQR 44.2 to 68.0 mg) with treatment, and 64.1 mg (IQR 47.8 to 71.4 mg) 1 mo after treatment. Factored for creatinine excretion, the placebo-treated patients had 44.7 mg/g at baseline and 41.5 mg/g with treatment, whereas the Oxadrop-treated patients had 28.8 mg/g at baseline and 26.5 mg/g with treatment.

The placebo-treated patients included two outliers with significantly higher urinary oxalate excretion than the other patients in either group (Figure 2); these two accounted for the higher mean values at baseline and after treatment in the placebo-treated groups. When we removed the two outliers, the oxalate excretions at baseline, treatment, and after treatment were comparable to the Oxadrop-treated group: 57.4 ± 17.8 mg (median 53.0 mg), 54.9 ± 12.0 mg (median 57.8 mg), and 52.2 ± 11.5 mg (median 54.4 mg), respectively. The individual responses of the study participants is shown in Figure 2. Exclusion of the two patients with significantly higher values for oxalate excretion did not affect the negative results by Kruskal-Wallis test or *t* test.

The dietary protocol, in which patients recorded and then attempted to replicate their diet during urinary collections, seemed to be effective in controlling other urinary variables. Urine volumes were similar in both the active and placebo preparations in all three periods. Urinary sodium remained relatively and statistically unchanged during the three periods. Although urea excretion was higher during treatment periods for both placebo- and active preparation–treated patients, the effect was not statistically significantly different. There were no differences in urine excretion of potassium, phosphate, calcium, sulfate, or magnesium (data not shown).

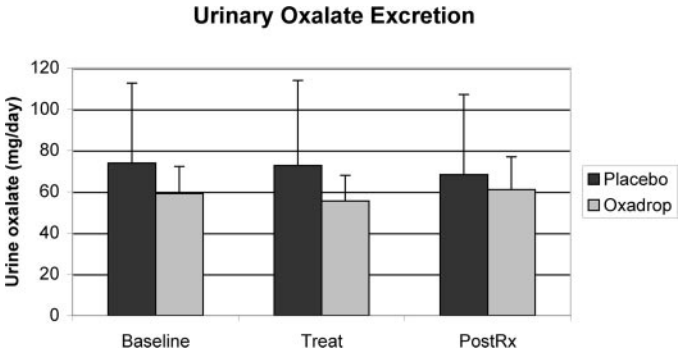

Figure 1. Twenty-four-hour urinary oxalate excretion (mg/d) in patients who took placebo and active preparation (Oxadrop) at three points: Baseline, treatment, and off-treatment (PostRx). $P = 0.89$ for placebo and 0.67 for Oxadrop (Kruskal-Wallis).

Adverse Events

No adverse events were noted in patients while on the study preparation. All randomized patients completed the study and were included in the statistical analyses. One participant reported mild diarrhea, and three reported mild constipation while taking placebo, whereas none reported these symptoms while taking the active preparation. One patient in the placebo group and one in the active group reported increased stool frequency. No patients reported nausea or diminished appetite.

In the poststudy questionnaire, patients were asked to attempt to identify in which group they had been. Eight of 10 patients in both groups answered the question. In both the placebo and active preparation groups, 75% (six of eight) of participants correctly identified their group.

Discussion

Previous studies suggested that lactic acid bacteria could effectively lower urinary oxalate excretion. Lactic acid bacteria are normal intestinal commensals, ubiquitous in fermented and nonfermented foods. They are present in unpasteurized yogurt, beer, and cheese. Oral lactic acid bacteria are classified by the Food and Drug Administration as "generally regarded as safe." They do not cause infections in healthy individuals when ingested orally.

This study was designed to follow up on an investigation reported by Campieri *et al.* (3). That study demonstrated that a variety of lactic acid bacteria species could degrade oxalate *in vitro*, if to a lesser degree than *Oxalobacter formigenes*. A preparation of these bacteria was administered to six participants with calcium stones and idiopathic hyperoxaluria for 30 d. All six participants experienced a nearly 50% reduction in urinary oxalate excretion. The reduction persisted after the preparation was no longer administered. In participants with enteric hyperoxaluria as a result of various bowel disorders, Oxadrop led to a reduction in urinary oxalate excretion of 19% when administered at a dosage of one packet per day and 24% when given twice a day (4). Whether the effects of these preparations in those two studies was due to direct oxalate degradation in the intestinal lumen was not determined.

We therefore performed a randomized, placebo-controlled trial of this same preparation in participants with idiopathic hyperoxaluria. Unlike the studies of Campieri and Lieske, we did not demonstrate a significant reduction in urinary oxalate attributable to lactic acid bacteria. The reason for this discrepancy in results is not known. The strength of our study, unlike the two other studies of this preparation, is that it was randomized and double-blinded. We also had participants duplicate their diets during the 24-h urine collections, a procedure that seems to have been effective, although one that has not been formally validated.

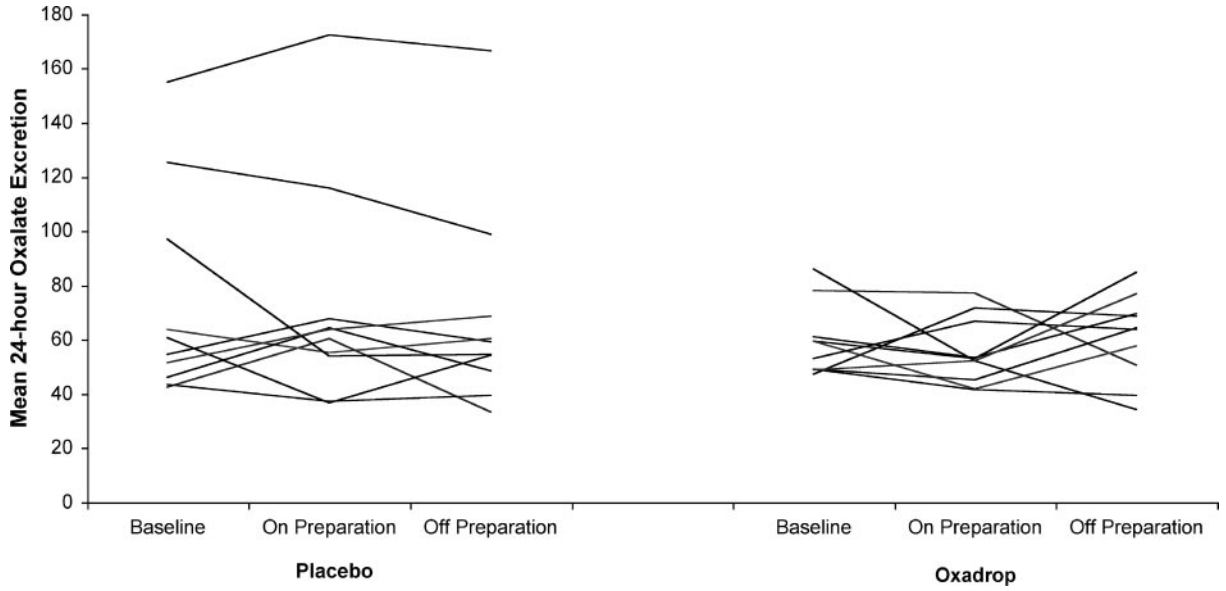

Figure 2. Individual responses of patients who took placebo and active preparation (Oxadrop) at three points: Baseline, treatment, and off-treatment (PostRx).

Potential limitations in our study include a question about timing of administration. As in the previous two studies, our instructions directed participants to take the preparation with the largest meal of the day. We have no measure of the participants' compliance. The importance of the timing of administration for efficacy is not clear, especially because in Campieri's study the effect persisted after administration of the study preparation ceased. We cannot be certain that the blinding was completely effective, because 75% of each group correctly guessed their assignment.

We could not ourselves confirm that the study preparation remained viable throughout the course of the study. The sponsor indicates that the shelf life of the preparation, a freeze-dried preparation of bacteria, extends to 2 yr when refrigerated, a period greater than that for which we held the study preparation in refrigerators in our research pharmacy.

The preparation is similar to that used in the first study of lactic acid bacteria for idiopathic hyperoxaluria, a study that showed a significant effect to reduce urine oxalate excretion. Four of the strains are identical but were mixed in Campieri's study in a ratio of 1:1:1:1, whereas Oxadrop contains *L. acidophilus*, *L. brevis*, *S. thermophilus*, and *B. infantis* mixed 1:1:4:4. All three studies used lactic acid bacteria prepared by the same manufacturer. This study's dosage was 10.8×10^{11} bacteria, not significantly more than that used in the other two studies, which was 8×10^{11} bacteria.

We instructed the participants to refrigerate the preparation as well and do not know whether they did so. Microbiologists at VSL Pharmaceuticals, the manufacturer, assure us that batch-to-batch consistency is maintained *via* a stringent quality control program and that each batch is grown from identical freeze-dried strains.

We might question whether we studied a group of participants with "metabolic" hyperoxaluria rather than a group with absorptive hyperoxaluria. Although the latter designation may apply to the participants with enteric hyperoxaluria studied by Lieske *et al.* (7) the utility of such a designation in studying participants who do not have either enteric hyperoxaluria or primary hyperoxaluria has not been established.

The mechanism by which lactic acid bacteria might reduce urinary excretion of oxalate remains unclear. The organism most studied in this role is not a lactic acid bacterium but the colonic anaerobe *O. formigenes*. This organism takes up oxalate from the intestinal lumen *via* an oxalate/formate exchanger encoded by gene *oxlT* (8). Oxalate is then metabolized by oxalyl-CoA decarboxylase, encoded by gene *oxc* (9). The lactic acid bacteria preparation that we studied was shown not to possess *oxlT*, *oxc*, or *frc* (3). Some lactic acid bacterium species do possess enzymes that are at least partially homologous to those demonstrated in *O. formigenes*. For instance, a strain of *Bifidobacterium lactis* isolated from yogurt showed oxalate-degrading activity *in vitro* and then was shown to have a gene 47% homologous with oxc from *O. formigenes*, which produced an active enzyme (10). Because lactic acid bacteria use carbohydrate as their main substrate, whereas *O. formigenes* metabolizes only oxalate, the relative contribution of any oxalate utilization by the former may be substantially less.

We do not know that the strain of *B. infantis* present in Oxadrop is similar in its oxalate-degrading properties to a strain of the same species that degraded 26.7% of a given amount of oxalate, compared with 60.6% for a *B. lactis* strain and 100% for *O. formigenes* (10). In that same study, another strain of *B. infantis* degraded only 4% of the oxalate in the culture medium, not much more than the negative control, *E. coli*. Another strain of *L. acidophilus* has the *frc* and *oxc* genes. It is interesting that their transcription occurred only when first adapted to subinhibitory concentrations of oxalate and then exposed to pH 5.5 (11). These requirements for gene transcrip-

tion may explain the failure to detect previously the genes in Oxadrop strains (3). These specific and necessary conditions may also represent obstacles in the use of these bacteria as therapeutic agents.

Another related preparation of lactic acid bacteria, made by the same manufacturer as Oxadrop, is VSL #3. VSL #3 is similar but not identical in its lactic acid bacteria selection to Oxadrop. VSL #3 was recently shown to be effective in degrading oxalate *in vitro*, although the specific strains that are responsible for this metabolism have not been determined (12). Lactic acid bacteria that are isolated from the feces of dogs and cats also degrade oxalate *in vitro* (13). Lactic acid bacteria could be useful for the lowering of urinary oxalate excretion, although the circumstances in which this therapy might be most efficacious have not been ascertained.

Conclusion

We did not confirm an effect of lactic acid bacteria on urinary oxalate excretion in people with idiopathic hyperoxaluria. Despite growing evidence in the scientific literature regarding the real basis for an effect of these organisms on oxalate metabolism, our trial yielded a negative result.

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Disclosures

J.A. is employed by Litholink Corp.

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