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A Double-Blind, Placebo Controlled, Randomized Phase 1 Cross-Over Study with ALLN-177, an Orally Administered Oxalate Degrading Enzyme

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Abstract

Background: Hyperoxaluria may result from increased endogenous production or overabsorption of dietary oxalate in the gastrointestinal tract leading to nephrolithiasis and, in some, to oxalate nephropathy and chronic kidney disease. ALLN-177 is an oral formulation of a recombinant, oxalate specific, microbial enzyme oxalate decarboxylase intended to treat secondary hyperoxaluria by degrading dietary oxalate in the gastrointestinal tract, thereby reducing its absorption and subsequent excretion in the urine. **Methods:** This double-blind, placebo controlled, randomized, cross-over, phase 1 study of ALLN-177 evaluated the tolerability of ALLN-177 and its effect on urinary oxalate excretion in 30 healthy volunteers with hyperoxaluria induced by ingestion of a high oxalate, low calcium (HOLC) diet. The primary end point was the difference in the mean 24-hour urinary oxalate excretion during the ALLN-177 treatment period compared with the placebo treatment period. **Results:** The daily urinary oxalate excretion increased in the study population from 27.2 ± 9.5 mg/day during screening to 80.8 ± 24.1 mg/day (mean ± SD) on the HOLC diet before introducing ALLN-177 or placebo therapy for 7 days. Compared to placebo, ALLN-177 treatment reduced urinary oxalate by 11.6 ± 2.7 mg/day, $p = 0.0002$ (least squares mean ± SD). **Conclusions:** In healthy volunteers, with diet-induced hyperoxaluria treatment with ALLN-177, when compared to placebo, significantly reduced urinary oxalate excretion by degrading dietary oxalate in the gastrointestinal tract and thereby reducing its absorption. ALLN-177 may represent a new approach for managing secondary hyperoxaluria and its complications.

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Introduction

Hyperoxaluria is a serious metabolic condition that arises either from endogenous overproduction of oxalate in the liver (primary hyperoxaluria types I–III; OMIM 259900, 260000, and 613616) [1] or from over-

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absorption of oxalate in the gastrointestinal tract, either from bowel disease or from as yet other undefined causes, all termed secondary hyperoxaluria [2, 3]. Kidney stones are the most common clinical abnormality that arises from secondary hyperoxaluria. Epidemiologic data suggest that there is a continuum of risk, so that with increasing levels of hyperoxaluria, there is an increasingly greater risk of kidney stone formation [4]. Furthermore, recent epidemiologic studies link kidney stone recurrence to the development of chronic kidney disease (CKD) [5]. Additional complications in some patients with secondary hyperoxaluria may include oxalate nephropathy, nephrocalcinosis and progression to end-stage renal disease and damage to the transplanted kidney if present [6–8].

Recent guidelines for the prevention and management of nephrolithiasis do not contain recommendations for pharmacologic therapy, due to the absence of such options [9, 10]. To date, diets with a low oxalate, salt and protein coupled with high water intake is viewed as the primary therapy for secondary hyperoxaluria, but compliance with and effectiveness of these measures are poor [10–12]. Furthermore, given the ubiquity of oxalate in many healthy foods (green vegetables, nuts, chocolate, grains, fruits, etc.) and its absorption-dependent increase with a high salt, high fat and low calcium content diets typical for Western civilizations, it is challenging for most patients to maintain sufficient dietary oxalate restrictions successfully over time [13]. Therefore, it is not surprising that hyperoxaluria remains a cause of repetitive kidney stone formation [14, 15]. Thus, a pharmacologic approach to lessen hyperoxaluria is needed to help reduce nephrolithiasis and its related morbidities.

ALLN-177 is being developed as an oral, substrate-specific enzyme therapy that degrades oxalate along the gastrointestinal tract. Here, we report results of a first proof-of-principle study of ALLN-177 in humans. This was a double-blind, placebo controlled, randomized crossover study of ALLN-177 in healthy volunteers with diet-induced hyperoxaluria.

This study in healthy volunteers with hyperoxaluria induced by consumption of a controlled high oxalate, low calcium (HOLC) diet enabled a test of the ability of ALLN-177, an oral enzyme therapy, to degrade dietary oxalate in the gastrointestinal tract. The demonstration of the tolerability of ALLN-177 and its ability to reduce urinary oxalate excretion in this experimental setting in healthy volunteers provided critical preliminary data to allow subsequent clinical studies to be started in patients with secondary hyperoxaluria and nephrolithiasis.

Materials and Methods

ALLN-177

ALLN-177 is an orally administered recombinant oxalate decarboxylase enzyme obtained from *Bacillus subtilis* expressed in *Escherichia coli*. It is a manganese-dependent enzyme that catalyzes the conversion of oxalate to formate and carbon dioxide. After purification and crystallization, ALLN-177 is filled into capsules (1,500 units/capsule). One unit of enzyme activity is defined as the amount of enzyme required to convert 1 μmol of oxalate into formate in 1 min at 25°C and pH 4.0.

Participants

After providing informed consent, 33 healthy male and female volunteers were enrolled, who were between 18 and 58 years of age and had the following characteristics: body mass index of 18–29.5 kg/m^2 and whose 24-hour urinary oxalate excretion was <45 mg/g creatinine. Furthermore, all patients had a 24-hour urinary calcium excretion <300 mg and a normal 24-hour urinary citrate excretion when indexed for urinary calcium [16]. The study was approved by the Sterling Institutional Review Board, Atlanta, Ga., USA.
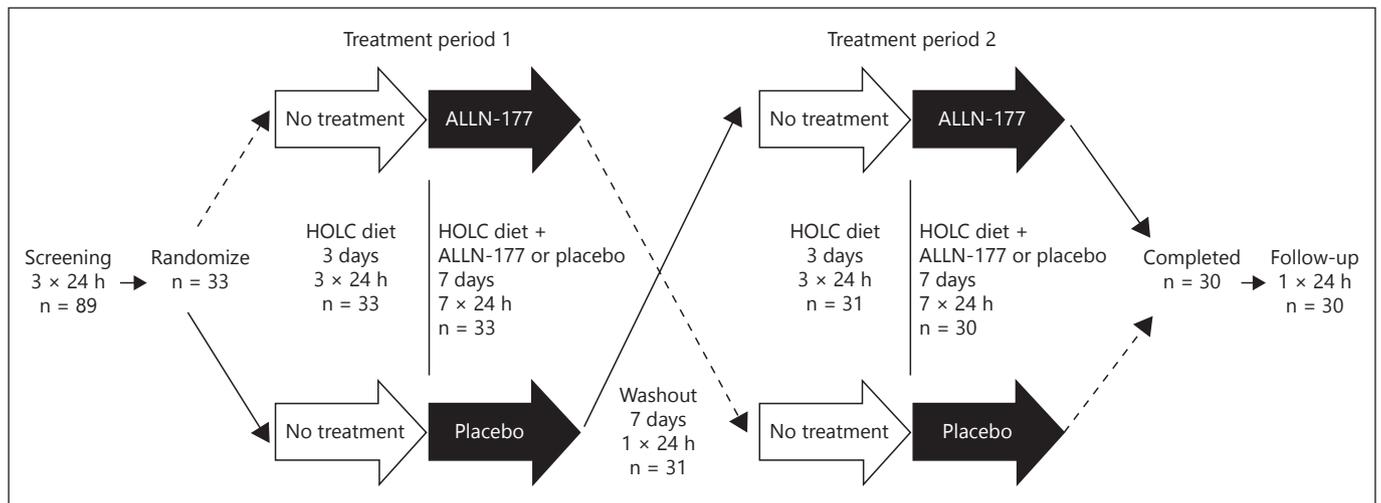
Study Design

The study was conducted at a single inpatient research unit. The study included a 24-day screening period, two 10-day inpatient treatment periods on the HOLC diet (comprised of a 3-day induction period and 7-day treatment period), separated by a 1-week outpatient washout phase while on their usual diet, to eliminate a potential carryover effect of the study drug, and a follow-up visit 7 days after treatment period 2 (fig. 1). Subjects were randomized to either ALLN-177 (7,500 units/meal, 3 times/day for a total of 22,500 units/day) or matching placebo. Placebo capsules were identical to ALLN-177 in size, color, appearance and similar in weight. After the washout phase, subjects began treatment period 2 and were crossed over to their opposite treatment (placebo or ALLN-177).

To induce and maintain hyperoxaluria, subjects consumed a controlled HOLC diet (1,000 mg oxalate; 400 mg calcium; online suppl. table 1a, b; for all online suppl. material, see www.karger.com/doi/10.1159/000448766) during each 10-day treatment period. The diet was developed by a registered dietitian (RD) with menu development software using nutrient values from the USDA National Nutrient Database for Standard Reference and was prepared by the Research Kitchen under the direction of the RD. Each individual's energy needs were calculated to the nearest 100 kcal using the Harris–Benedict equation plus a standard daily activity factor of 1.3 kcal/day. Women were provided with 2,000 kcal and men with 3,000 kcal base menus. The 3 meals in the 2-day rotating menu were served in a controlled, monitored dining setting; additional low-oxalate snacks and measured amounts of water were provided. The menus contained a variety of high oxalate foods, such as spinach pesto, a rhubarb and berry smoothie, vegetable soup with chopped spinach, among others.

Urinary Biochemistry Measurements

The efficacy of ALLN-177 was evaluated based on 24-hour urinary oxalate excretion. The University of Texas Southwestern Charles and Jane Pak Center for Mineral Metabolism and Clinical Research in Dallas TX analyzed the 24-hour urine samples. Oxa-

Fig. 1. Study design: during a 24-day screening period, 3 outpatient 24-hour urines were collected, of which 2 were consecutive 24-hour urines. After randomization, daily 24-hour urines were collected during 3 days of HOLC diet periods, and the 7 day treatment periods. One 24-hour urine was collected, each in the middle of the washout and at the end of the 7-day follow-up period. During first 3 days after randomization and after crossover, participants consumed only the HOLC diet. In treatment period 1 or 2, participants were randomized to either ALLN-177 (7,500 units/meals, 3 times/day for a total of 22,500 units/day) or matching placebo starting from day 4 through day 10 of each treatment period and they continued to consume the HOLC diet.

late concentration was measured using a sensitive HPLC Dionex method as previously described [16]. Urinary calcium and magnesium levels were measured using atomic absorption spectrophotometry, and Cobas Mira plus analyzer was used for measurement of creatinine, citrate and uric acid in the same laboratory [16].

Primary Study Outcome

The primary end point of the study was the within-subject difference in oxalate excretion in the mean 24-hour urinary oxalate excretion (mg/day) during the last 4 days of each 7-day treatment period (mean excretion while on placebo versus mean excretion while on ALLN-177).

Safety Analyses

The safety assessments included treatment emergent adverse events (TEAEs), changes in and abnormal findings in clinical laboratory parameters and vital signs, physical examinations and ECGs.

Statistical Analysis

Raw data summaries are reported as the mean ± SD. Least squares (LS) means are presented with their SE. Initially, the planned study size was 40–60 subjects. Since this was a phase 1 study, the magnitude of the effect size of ALLN-177 was unknown and the variability of urinary oxalate levels on the HOLC diet could only be estimated. Therefore, an interim analysis was performed on an initial cohort of 15 subjects by an unblinded statistician and included both a futility analysis and a conditional power analysis to determine the final sample size needed to complete the study. It was determined that the probability of rejecting the null hypothesis was >0.999, and therefore, a total of 30 subjects would be sufficient to demonstrate statistical significance for the primary end point. Other than the calculated probability of rejecting the null hypothesis and the final study size determination, all data remained blinded to all the authors until the study was completed.

All statistical analyses were performed after the last subject completed the follow-up visit. A mixed model with random effects for crossover designs was used for the analysis. Two response values were included for each subject: the observed mean of 24-hour urine oxalate excretion for the last 4 days of ALLN-177 treatment and the observed mean for the last 4 days of placebo treatment. Included in the final statistical model were the categorical effects: treatment, sequence and period. Subject nested within sequence were included as a random effect. Also included in the analysis was the treatment by baseline interaction. Significance was based on the LS mean difference in the response between ALLN-177 and placebo. The null hypothesis was to be rejected if the p value was <0.048.

Results

Thirty-three healthy volunteers were enrolled in the study, and 30 completed the study (table 1). Sixteen of 33 participants (48.5%) were assigned to ALLN-177 in treatment period 1, and 17 of 33 (51.5%) were assigned to ALLN-177 in treatment period 2. Three participants withdrew from the study, 2 after treatment period 1 (both were in the placebo arm) and 1 in treatment period 2 (in the ALLN-177 arm during the period on the HOLC diet, before study drug administration). The first

Table 1. Baseline subject characteristics

Parameters	Value
Age, years	39.7 (10.7)
BMI, kg/m^2	24.9 (3.1)
Gender, n (%)	
Male	27 (81.8)
Female	6 (18.2)
Race, n (%)	
White/Caucasian	28 (84.8)
Black/African American	3 (9.1)
Other	2 (6)
Urine volume, l/day	1.6 (0.7)
Oxalate, mg/day	27.2 (9.5)
Oxalate, mg/g creatinine	21.1 (7.0)
Calcium, mg/day	135.1 (56.7)
Citrate, mg/day	460.4 (182.4)
Magnesium, mg/day	93.0 (31.8)
Uric acid, mg/day	427.7 (145.6)
eGFR, ml/min/1.73 m^2	102.3 (14.5)

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

2 participants withdrew from the study for personal reasons, unrelated to the study drugs, and the third one was withdrawn due to an inter-current infection. There was 100% compliance with both study drug and meal consumption.

The daily urinary oxalate excretion (mean ± SD) for all subjects from the study increased from 27.2 ± 9.5 mg/day at screening to 80.8 ± 24.1 mg/day while consuming the HOLC diet during the induction period before study drug (fig. 2a; p < 0.0001). The lowest individual increase in urinary oxalate excretion while on the HOLC diet was 9.4 mg/day (from a mean screening value of 36.6 to 46.0 mg/day) and the greatest individual increase was to 127.3 mg/day (from a mean screening value of 25.0 to 152.3 mg/day). There were no untoward negative effects on the study participants from the consumption of the HOLC diet before or during either study drug administration.

The mixed model analysis with random effects for crossover design demonstrated that oxalate excretion on ALLN-177 was significantly reduced compared with placebo (LS mean ± SE difference of 11.6 ± 2.7 mg/day, p = 0.0002). During the last 4 days of placebo treatment, participants excreted a mean ± SD of 80.5 ± 27.4 mg oxalate/day, compared with 67.2 ± 18.8 mg oxalate/day during the ALLN-177 treatment periods (p = 0.0002), thereby achieving a 17% reduction in mean urinary oxalate excretion with ALLN-177 (mean reduc-

tion 12.5 ± 16.5 mg/day, p = 0.0002) with ALLN-177 (fig. 2b).

The effect of ALLN-177 to reduce urinary oxalate did not differ by treatment period allocation. However, as seen in figure 2c and table 2, individual responses to the effect of ALLN-177 versus placebo varied considerably among the study participants, ranging from no change in urinary oxalate up to a reduction of 62.4 mg/day. Sixteen subjects (53%) had reductions in urinary oxalate excretion ≥10 mg/day. When viewed as a percentage change in urinary oxalate excretion from ALLN-177 compared to placebo, 5 subjects had ≥30% reduction, 10 had ≥20% reduction and 18 subjects had ≥10% reduction (table 2). A post hoc analysis showed that 18 subjects with >10% reduction in urinary oxalate excretion had higher mean oxalate levels during induction on the HOLC diet compared with 12 subjects who did not (89.5 ± 27.0 vs. 68.4 ± 14.9 mg/day, p = 0.005) and greater increases in oxalate excretion from screening to induction (mean increase 62.8 ± 26.5 vs. 40.7 ± 19.4 mg/day, p = 0.007).

The significant reduction in oxalate excretion while subjects were on the HOLC diet during treatment with ALLN-177 compared to treatment with placebo was not associated with any significant difference in the measured urinary calcium, citrate, magnesium, uric acid, pH and urinary volume (online suppl. table 2). In addition, the baseline urinary calcium value while subjects were on their free living diet and were not enrolled into study was reduced from 135 ± 56.7 to 89.8 ± 38.1 mg/day while subjects were in the study consuming HOLC diet regardless of treatment assignment, with a net reduction of 47 mg/day. In the single 24 h urine collected from each participant during the follow-up period, 7 days after the study was completed, the mean 24-hour urine oxalate excretions were not different from the screening values (follow-up: 32.73 ± 16.49 mg/day versus screening: 27.2 ± 9.5 mg/day), indicating that there was no carryover effect of the drug within days of its discontinuation.

There was no apparent drug-related trend in TEAEs and there were no serious adverse events during the study. One subject was removed from the study by the research center site principal investigator due to inter-current infection that was found on study day 3, prior to the treatment period 2 and study drug administration. Fourteen subjects had a TEAE across all study periods; only one of these (diarrhea) was judged as possibly related to study drug (placebo). The largest number of TEAEs was in the gastrointestinal system (n = 6), evenly distributed between placebo and ALLN-177. All TEAEs had resolved by the end of the study.
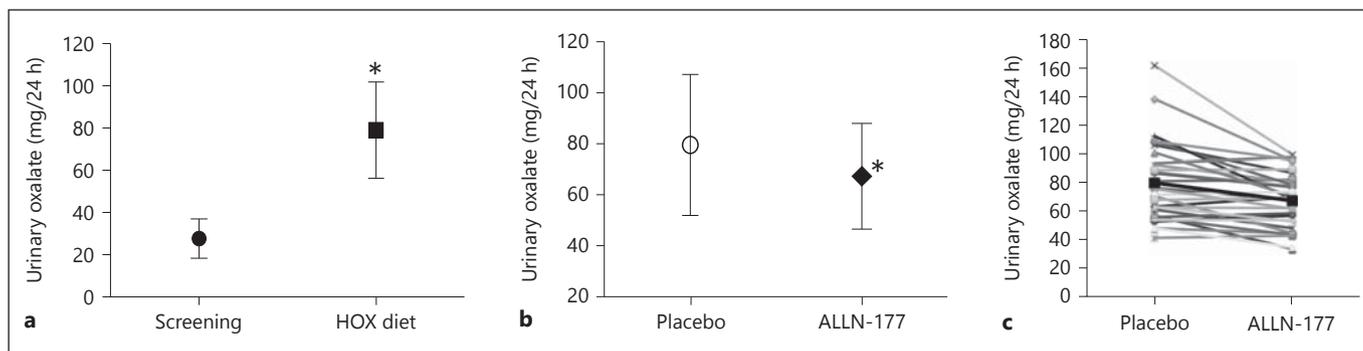
Fig. 2. a Urinary oxalate excretion on HOLC diet. The difference between the urinary excretion (mean ± SD) of oxalate during 3 screening urine collections while subjects were consuming a free living diet and induction days (mean of days 2 and 3 in treatment periods 1 and 2) on the HOLC diet. The mean urinary oxalate excretion was increased from 27.2 ± 9.5 mg/day at screening to 80.8 ± 24.1 mg/day while on HOLC diet during the induction period before study drug administration (* p < 0.0001) **b** The effect of ALLN-177 versus placebo on urinary oxalate excretion. The difference between the 24-hour urinary excretion (mean ± SD) of oxalate during the last 4 days of the treatment with placebo (O) and ALLN-177 (♦), while consuming the HOLC diet. The mean urinary oxalate excretion on ALLN-177 was 12.5 ± 16.5 mg/day lower compared to placebo treatment (* p = 0.0002). **c** Individual changes with ALLN-177 therapy. The individual changes (n = 30) during the last 4 days of placebo treatment versus ALLN-177 treatment. The LS mean value during each treatment period is denoted by the black (■) and connected by the black line (—).

Discussion

This double blind, placebo controlled, randomized crossover study in healthy volunteers with diet-induced hyperoxaluria serves as 'proof of principle' that ALLN-177, an oral and substrate specific enzyme-based therapy, can produce a significant and prompt reduction in 24-hour urinary oxalate excretion, while being well tolerated. The mean reduction of 11.54 mg/day was seen across the entire group of healthy volunteers. Moreover, in a majority of the study population (18 of 30), the mean reduction was 20.4 mg/day, ranging from 8.3 to 43.7 mg/day. Furthermore, such data are crucial in support of progression to further clinical studies in patients with secondary hyperoxaluria and kidney stones.

Dietary hyperoxaluria, as simulated by the current study, is well recognized as an important feature of recurrent kidney stones in several populations, including idiopathic stone formers, and in those with enteric hyperoxaluria related to various gastrointestinal pathologies, especially after Roux-en-Y bariatric surgery procedures [2, 3, 5, 6]. In the more severely affected individuals, this may lead to oxalate nephropathy, which if progressive leads to CKD and end-stage renal disease that would require dialysis or kidney transplantation [7]. To date, there are no prospective clinical trials on the efficacy of diet for reduction in secondary hyperoxaluria and clinical events, such as kidney stones. Borghi

et al. [17] studied the effect of 2 dietary approaches in patients with idiopathic hypercalciuria, concomitant hyperoxaluria and frequent stone formation, on subsequent kidney stone formation. Using a normal calcium diet that was also protein-modified and salt-restricted, compared to a low calcium intake diet alone, there was a significant reduction in stone formation over 5 years in the former group (where 12 of 60 participants formed stones compared to 23 of 60 in the latter group), and that was associated with a 7.2 mg/day reduction in urinary oxalate excretion. Based largely on that study, and supported by epidemiologic evidence that higher quartiles of urinary oxalate excretion were associated with higher frequency of kidney stone formation [4], the approach to patients with secondary hyperoxaluria and frequent kidney stones has been to limit dietary oxalate and increase fluid intake. Therefore, we posit that in those who form stones primarily due to secondary hyperoxaluria, sustained reduction in urinary oxalate excretion over time may reduce the number of stone episodes.

Such approaches in subsequent studies have included short-term and not well-controlled studies [10–12]. Oxalate is ubiquitous in many foods in the American diet [18, 19], and it is not clear that patients outside of a clinical trial can easily adhere to one of the several recommended diets that might reduce urinary oxalate, either due to availability of oxalate-free foods, lifestyle or the econom-

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Margolin/Brettman

Table 2. Urinary oxalate during screening, then on HOLC diet during induction period and change between placebo and ALLN-177 treatment periods

Subject ID (n = 30)	Urinary oxalate at screening[1], mg/24 h	Urinary oxalate HOLC oxalate diet[2], mg/24 h	Urinary oxalate (ALLN-177 minus placebo)[3]	
			mg/24 h	%
21	21.19	56.60	−23.55	−41.08
55	25.46	101.20	−43.70	−38.87
23	24.98	144.50	−62.35	−38.51
27	19.47	72.90	−26.02	−36.75
85	22.66	117.40	−43.27	−31.28
70	14.07	101.80	−30.13	−29.85
89	20.70	55.00	−13.55	−28.00
52	22.63	106.00	−30.20	−27.76
42	30.35	63.70	−12.03	−21.70
26	19.40	59.40	−13.23	−21.55
84	23.16	66.60	−14.05	−18.50
47	35.33	116.80	−19.23	−18.09
83	40.87	70.60	−10.60	−16.69
50	16.31	92.20	−11.95	−12.94
81	60.97	130.10	−12.77	−11.75
61	24.73	78.50	−10.20	−11.64
34	18.78	108.70	−9.84	−11.31
72	35.12	74.10	−8.30	−10.86
39	25.63	52.80	−3.98	−8.22
43	25.67	69.90	−0.67	−1.06
60	36.11	54.30	0.13	0.24
64	23.46	100.20	0.75	0.84
19	22.19	66.50	0.70	0.86
86	15.01	64.60	1.45	2.13
80	35.30	80.80	2.17	3.03
79	36.63	46.30	1.55	3.74
56	16.59	86.20	4.25	4.53
40	35.90	64.70	2.85	5.10
41	23.47	66.60	3.85	7.26
16	35.90	68.80	7.02	11.09

[1] Mean of urinary oxalate during screening while subjects were on the free living diet.
[2] Mean of urinary oxalate while on HOLC diet during induction period before study drug administration.
[3] Values represent the difference between placebo and ALLN-177 treatment periods, percent differences are calculated as 100 × (mean placebo − mean ALLN-177).

ic costs of using such diets [13]. Additionally, methods of food preparation may affect the soluble oxalate content as well, such that even the same food prepared differently may yield different oxalate burdens to patients [20]. Importantly, even with dietary counseling and adherence to a recommended diet, many patients with hyperoxaluria after bariatric surgery, or with bowel disease, remain with hyperoxaluria and develop stones and CKD [6, 21]. Other therapeutic approaches to reduce hyperoxaluria in patients with kidney stones have been evaluated. In the initial open-label study with a probiotic from lactic acid bac-

teria, a high dose of that compound in 6 patients with idiopathic calcium-oxalate nephrolithiasis resulted in a reduction in urinary oxalate excretion of about 15% on a random outpatient diet [22]. However, in a similar, non-randomized study, a probiotic given to patients with enteric hyperoxaluria had a transient effect in lowering urinary oxalate, and the urinary oxalate values returned to pre-treatment values at 3 months of continued treatment [23]. Furthermore, no change in urinary oxalate was observed in 10 patients with enteric hyperoxaluria who were enrolled in a non-randomized, open-label study of the

dietary phosphate binder, sevelamer hydrochloride, 3,200 mg t.i.d. for 7 days [24]. Lastly, in a study of patients with hyperoxaluria and kidney stones who were treated initially with a controlled, low oxalate diet, urinary oxalate did not further decrease after 4 weeks of treatment with probiotics [25]. All of these studies performed in patients with secondary hyperoxaluria did not evaluate stone prevention or recurrence.

In our study, a controlled HOLC diet in healthy volunteers was used to produce hyperoxaluria to the range seen in patients with either idiopathic or enteric hyperoxaluria or kidney stones [26]. The high oxalate diet utilized in this study contained around 3–5-fold more oxalate and about half of the calcium of a normal diet [27, 28]. The diet was selected to maximize dietary oxalate absorption and provide a stringent test of the ability of ALLN-177 to degrade dietary oxalate in this experimental setting.

The study demonstrated that orally administered ALLN-177 significantly reduced oxaluria, without any pattern of treatment-limiting side effects. Importantly, no other measured urinary parameters were changed by ALLN-177 therapy, and in particular, there were no changes in urinary calcium, citrate or uric acid levels, urinary volume or pH when compared to placebo treatment. It was reasoned that if ALLN-177 therapy could reduce urinary oxalate excretion in the face of this high dietary oxalate load, then the prospect of reducing dietary oxalate absorption in patients with secondary hyperoxaluria would be promising.

There were 18 subjects (60%) with >10% reduction in urinary oxalate excretion; interestingly, these 18 subjects had a higher mean 24 h urine oxalate excretion during the induction period on the HOLC diet and a greater increase from screening to induction compared with the 12 subjects who did not have a reduction in oxalate excretion with ALLN-177 (around 21 mg/day difference between these 2 groups). These data suggest that some of the healthy subjects who absorbed dietary oxalate more efficiently and therefore had higher levels of hyperoxaluria had a greater reduction in urinary oxalate excretion with ALLN-177 therapy. Patients with secondary hyperoxaluria may share this trait [29, 30], and thus may respond to ALLN-177 to a similar or greater extent than observed in this trial. This idea will be evaluated in subsequent clinical trials.

Other possible explanations for the variability in the effect of ALLN-177 on urinary oxalate excretion might include differences in stomach pH (which affects release of soluble oxalate from food), differences in sites within the gastrointestinal tract for oxalate absorption and colonization with oxalate degrading bacteria.

At present, the exact site of oxalate absorption in humans remains uncertain, but likely includes sites in both the proximal and the distal gastrointestinal tract [31, 32]. The primary site of action of ALLN-177 is thought to be in the stomach and upper small intestine, as demonstrated in a pre-clinical porcine model of diet-induced hyperoxaluria (data not shown). However, ALLN-177 is stable throughout the gastrointestinal tract, and retains enzymatic activity in the distal intestine as demonstrated in a rodent model [33]. Thus, we cannot rule out distal effects in the colon in our healthy subjects given ALLN-177.

Our study had some limitations. Most kidney stone formers with hyperoxaluria do not eat a diet with as much oxalate or as little calcium as we employed in this study. However, as discussed above, we felt that if ALLN-177 could reduce dietary oxalate absorption under such conditions, subsequent clinical studies in patients would be justified. In our study, we did not measure all the necessary urinary constituents to allow for a formal calculation of calcium-oxalate supersaturation indices, a parameter often used to estimate the urinary saturation of stone forming salts and to gauge the propensity for stone formation. However, since there were no changes in what we did measure, except oxalate, it seems likely that there would not be an increase in calcium-oxalate supersaturation, but rather a decrease. However, we await studies in kidney stone formers with hyperoxaluria treated with ALLN-177 for this demonstration. We did not interrogate the timing within the 24-hour period of the effect of ALLN-177 to lower urinary oxalate, as we only collected complete 24-hour urines. This might be useful in subsequent studies of patients with kidney stones due to secondary hyperoxaluria. We also did not determine the colonization status of our participants with respect to *Oxalobacter formigines*, which is thought to be a factor in limiting dietary oxalate absorption [33]. The study was performed in a single research center on healthy volunteers, and perhaps, if performed in a real world environment on stone patients, the results might differ. Lastly, we studied only one dose of ALLN-177, and perhaps, a different dose would be more effective for some individuals.

This phase 1 study was designed to demonstrate proof of efficacy that oral therapy with ALLN-177, when taken with meals, can reduce urinary oxalate excretion in the experimental setting when healthy volunteers consumed a HOLC diet. The study achieved that goal.

Although exposure in the current study to ALLN-177 was only 7 days, and the drug was well tolerated without any treatment-related safety issues. As it is formulated to specifically digest oxalate in the in the gastrointestinal tract and is not absorbed as an intact molecule (data not shown), it is expected to remain safe with longer term use in subsequent trials.

In summary, when using one specific dose of ALLN-177 in a group of healthy volunteers with diet-induced hyperoxaluria, there was a significant reduction in urinary oxalate excretion compared to placebo in this randomized, double-blind placebo controlled single cross-over study. The onset of action was rapid and the drug was well tolerated. It seems likely that if efficacy of ALLN-177, as demonstrated in healthy volunteers in the current study, is observed in patients with secondary hyperoxaluria, it might produce a clinically meaningful effect in reduction of hyperoxaluria and subsequent stone burden, but such studies and proof wait for the near future. The studies to evaluate the effect of ALLN-177 in patients with secondary hyperoxaluria and kidney stones are underway.

Acknowledgments

Disclosure Statement

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