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Efficacy and safety of *Oxalobacter formigenes* to reduce urinary oxalate in primary hyperoxaluria

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Abstract

Background. Primary hyperoxaluria (PH) is a rare genetic disease, in which high urinary oxalate (Uox) cause recurrent kidney stones and/or progressive nephrocalcinosis, often followed by early end-stage renal disease, as well as extremely high plasma oxalate, systemic oxalosis and premature death. *Oxalobacter formigenes*, an anaerobic oxalate degrading bacterium, naturally colonizes the colon of most humans. Orally administered *O. formigenes* (Oxabact) was found to significantly reduce urine and plasma oxalate. We aimed to evaluate its effect and safety in a randomized, double-blind, placebo-controlled multicenter study.

Methods. Oral Oxabact was given to PH patients (>5 years old, Uox > 1.0 mmol/1.73m^2/day, glomerular filtration rate (GFR) > 50 mL/min) at nine PH referral sites worldwide. Primary endpoint was the change from baseline in Uox (mmol/1.73m^2/day) after 24 weeks of treatment (>20% reduction).

Results. Of the 43 subjects randomized, 42 patients received either placebo (23 subjects) or Oxabact (19 subjects). The change in Uox was <20% and not different between groups (P = 0.616). *Ad hoc* analysis was performed in 37 patients compliant with medication and urine processing. Change in Uox was −19% in subjects given

Oxabact and −10% in placebo, (P = 0.288), but −21 and −7% with Uox expressed as molar creatinine ratio (Ox:Cr, mmol/mol, P = 0.06). Reduction of Ox:Cr was more obvious for patients with higher baseline values (>160 mmol/mol, Oxabact −28%, placebo −6%; P < 0.082). No serious adverse events were reported.

Conclusion. Oxabact was safe and well tolerated. However, as no significant change in Uox was seen, further studies to evaluate the efficacy of Oxabact treatment are needed.

Keywords: efficacy; *Oxalobacter formigenes*; primary hyperoxaluria; safety; treatment

Introduction

The primary hyperoxalurias (PH) types I, II and III are autosomal-recessive inherited diseases characterized by markedly elevated urinary oxalate excretion leading to recurrent urolithiasis, progressive nephrocalcinosis and all too often early renal failure [1]. Of the three forms, PH I is more severe, but its clinical, biochemical and genetic heterogeneity is marked, with some patients presenting already in infancy with end-stage renal failure and others experiencing only occasional passage of stones in adult life with maintained renal function [2, 3]. The median age when patients with PH I exhibit their first symptom is 5 years [4].

Although all PH forms are associated with overproduction of oxalate, they differ in the underlying metabolic dysfunction. In PH I, deficiency and/or mistargeting of hepatic alanine:glyoxylate-aminotransferase (AGT) results in metabolic overproduction of oxalate and glycolate [1]. In PH II, deficiency of the D-glycerate dehydrogenase/glyoxylate reductase results in metabolic overproduction of oxalate and glycerate [5]. Recently, mutations in a previously uncharacterized gene *DHDPSL* was found to cause a third type of PH [6]. *DHDPSL*, located on chromosome 10, is the gene encoding 4-hydroxy-2-oxoglutarate aldolase.

PH is rare but its incidence may be underestimated due to delayed diagnosis or the disease being overlooked. Surveys of nephrologists and urologists in France, Switzerland and The Netherlands have estimated the prevalence of PH type I, the most prevalent form, to be in the range of 1–2 cases per million people with an average incidence rate of 0.1–0.15/10^6/year [7–9].

When chronic renal insufficiency occurs, marked hyperoxalemia results in systemic deposition of calcium oxalate (oxalosis) with multiple organ system involvement which may include bone disease, erythropoietin refractory anemia, skin ulcers, digital gangrene, cardiac arrhythmias and cardiomyopathy [3]. These complications contribute significantly to the morbidity and mortality associated with PH. Patients requiring dialysis or who have extensive tissue oxalosis have a lower survival rate.

The severity of the hyperoxaluria correlates with renal outcome in patients with PH [10]. However, the only medical treatment currently available to reduce urine oxalate is pyridoxine, which is effective in fewer than half of the patients with PH type I and ineffective in types II and III [3, 11, 12]. Most current therapies for PH are directed to increase the urinary solubility of calcium oxalate to preserve renal function, including treatment with citrate, orthophosphate and magnesium but their efficacy has not been well characterized [3, 13, 14]. Thus, there is a need for an effective therapy to reduce urinary oxalate levels in patients with PH.

Oxalobacter formigenes is a strict anaerobe and a beneficial microbe, which relies exclusively on oxalate as a substrate to obtain energy [15]. Very little is known about when and how individuals become colonized or the persistence of *O. formigenes* over time. It is part of the normal intestinal flora in humans and it is non-pathogenic [16, 17]. The rate of colonization in healthy population has been reported to range from 40 to 77%. The bacterium is known to be susceptible to certain antibiotics and its colonization has been reported to be lower in populations at risk of higher antibiotic use and with recurrent kidney stones [18].

In rat models, there is evidence that *Oxalobacter* directly interacts physiologically with colonic epithelium to induce enteric oxalate elimination [19, 20]. In the mouse *agxt* knockout model of PH I, endogenous hepatic production accounts for excess oxalate excreted in the urine. Two independent studies noted a striking reduction in the degree of hyperoxaluria and renal injury due to oxalate in *agxt* knockout mice that were fed *O. formigenes* [21, 22]. Enhanced enteric elimination would be expected to reduce the levels of urinary oxalate in all types of PH.

The safety and efficacy of *O. formigenes* in PH have been investigated in two open-label studies in 13 subjects where either a frozen cell paste (OC2, live cells at $>10^{10}$ colony-forming units, CFU) or Oxabact™ (OC3), i.e. lyophilized *O.*

Table 1. Demographics and hyperoxaluria history of the study population

	Oxabact, $N = 19$	Placebo, $N = 23$	Total, $N = 42$
Sex, *n* (%)			
Male	11 (57.9)	8 (34.8)	19 (42.3)
Female	8 (42.1)	15 (65.2)	23 (54.8)
Ethnic origin, *n* (%)			
Caucasian	14 (73.7)	21 (91.3)	35 (83.3)
Black/African-American	0 (0.0)	0 (0.0	0 (0.0)
Asian	3 (15.8)	2 (8.7)	5 (11.9)
Other	2 (10.5)	0 (0.0)	2 (4.8)
Age at screening (years), mean (SD)	13.4 (6.47)	14.4 (6.99)	14.0 (6.70)
Primary hyperoxaluria, *n* (%)			
Type I	16 (84.2)	19 (82.6)	35 (83.3)
Type II	3 (15.8)	4 (17.4)	7 (16.7)
Time since diagnosis (years), mean (SD)	7.3 (5.8)	9.4 (6.0)	8.5 (5.9)
Estimated GFR (mL/min per 1.73m^2), mean (SD)	121.5 (44.58)	105.2 (29.97)	113.3 (37.6)
Genetics, *n* (%)			
Missense/other	2 (10.5)	5 (21.7)	7 (16.7)
Missense	6 (31.6)	6 (26.1)	12 (28.5)
Splice site	1 (5.3)	1 (4.3)	2 (4.8)
Splice/stop	0	2 (8.7)	2 (4.8)
Frameshift	1 (5.3)	5 (21.7)	6 (14.3)
Frameshift/other	5 (26.3)	3 (13.1)	8 (19.0)
Unknown	4 (21)	1 (4.3)	5 (11.9)

formigenes formulated in enteric-coated capsule (two capsules provided $>10^7$ CFU *O. formigenes*) was administered orally to subjects with PH twice a day for 4 weeks [21]. Results from these two studies showed that twice daily dosing was well tolerated and resulted in a statistically significant reduction of urinary oxalate levels [23]. Thus, Oxabact was found to be a potential new therapy for PH and was granted orphan drug status in the USA and EU.

The study reported here was performed to further investigate the efficacy and safety of an orally administered *O. formigenes* preparation [not less than (NLT) 10^7 CFU of Oxabact] given twice daily to reduce urinary oxalate in subjects with PH.

Materials and methods

A double-blind, randomized, placebo-controlled, multicenter, international clinical study was conducted to evaluate the safety and efficacy of *O. formigenes* administered as Oxabact in the reduction of urinary oxalate levels in subjects with PH. Within each geographic region (US and non-US), eligible subjects enrolled into the study were randomized (1:1) to receive orally either NLT 10^7 CFU of Oxabact or placebo twice daily with meals.

Subjects with a confirmed diagnosis of PH and were >5 years of age, with a urinary oxalate excretion >1.0 mmol/1.73m^2/day and with an estimated glomerular filtration rate (eGFR) ≥50 mL/min/1.73m^2 body surface area (BSA) were eligible for the study. Because of a potential effect on urine oxalate, PH I patients who were receiving pyridoxine medication had to be on a stable dose for at least 3 months prior to enrollment and maintained on the same dose throughout the study. Subjects who were not receiving pyridoxine at study entry were not allowed to initiate pyridoxine during study participation.

Subjects were stratified by renal function as defined by their estimated glomerular filtration rate (eGFR) at screening such that within each stratum (eGFR of >80 mL/min/1.73m^2 or subjects with an eGFR <80 mL/min/1.73m^2), there were an approximately equal number of subjects receiving Oxabact and placebo. Subjects received the study drug for 24 weeks. All subjects were monitored for safety throughout, with the last assessment at Week 24 in subjects continuing into an open-label extension study and at Week 28 in subjects who did not continue into the open-label extension study. All patients, including those on placebo in the double blinded study, were offered to participate in the open-label study, which, however, was voluntary. The safety under long-term exposure was examined within this follow-up analysis.

Fifty-eight subjects were screened for inclusion in the study, 43 of whom had a baseline mean urinary oxalate excretion exceeding 1.0 mmol/1.73m^2/day. The subjects were randomized, 20 to receive Oxabact (17 with PH I) and 23 (19 with PH I) to receive placebo. One patient randomized to Oxabact did not receive any study medication due to inability to swallow the capsules and was not included in study statistics. Treatment was initiated in 42 patients. Forty-one (95.3%) subjects completed the study, 18 (90.0%) in the Oxabact and 23 (100.0%) in the placebo group. One subject randomized to Oxabact withdrew consent after 18 days of treatment. Thus, two (4.7 %) subjects, both in the Oxabact treatment group, did not complete the study. The demographic data of the 42 patients in whom treatment was initiated is presented in Table 1.

Three patients were enrolled under a protocol inclusion waiver. The inclusion criterion waived in all three cases was renal function defined as an eGFR ≥50 mL/min/1.73m^2 BSA. One subject entered the study with an eGFR of 48 mL/min/1.73m^2, a second subject with an eGFR of 49 mL/min/1.73m^2 and the third with an eGFR of 43 mL/min/1.73m^2. All protocol waivers were approved prior to the subject receiving study medication.

Thirty-six subjects entered the open-label extension study, which primarily was done to examine safety issues during long-term administration of Oxabact.

Hyperoxaluria histories and eGFR (Table 1) as well as baseline urinary oxalate excretion rate and urinary oxalate to creatinine ratio were similar between treatment groups (Table 2, P = not significant.). Overall, 35 subjects (83.3%) had a primary diagnosis of PH I. The mean time following diagnosis was higher in the placebo treatment group (9.4 years) than in the Oxabact treatment group (7.3 years).

The primary endpoint of the study was percent change in urinary oxalate from screening to Week 24. Urinary oxalate was measured by three consecutive 24-h urine collections at screening (baseline, Week 0) and two consecutive collections at Weeks 4, 8, 12, 18 and 24. Plasma oxalate was determined at screening, Weeks 12 and 24. For *ad hoc* analyses, urinary oxalate was expressed as molar ratio in mmol/mol creatinine as well as 24-h excretion in mmol/1.73m^2/day.

Subjects were evaluated at the clinical study sites ($n = 9$) at screening and at Weeks 12 and 24 (and Week 28 if not participating in the open-label study) or termination. At study entry, subjects were provided local language diaries and were instructed to record daily the date and time they took the study drug and the approximate fluid intake. In addition, subjects were instructed to record any adverse events (AE) that occurred, any changes in concomitant medications and the dates of 24-h urine collections. The site coordinators contacted subjects weekly by telephone to assess drug compliance and any adverse events, to remind subjects to complete their daily diary entries, mail completed copies of diary pages, return unused study drug product to the clinical site on a monthly basis, to complete their 24-h urine collections and, for German subjects only, to collect their stool samples.

Subjects were given detailed instructions for the collection of 24-h urine samples at home. They were asked to keep the urine samples refrigerated until they were collected via a courier service and delivered to the site laboratories under refrigerated conditions. The sites were asked to bring the urines to room temperature, record the total volume, prepare appropriate aliquots preserved both with 6 N HCl, as well as with toluene, and ship them to a central laboratory (Mayo Clinic Laboratories for clinical trials (MCLCT), Rochester, MN) for testing. Urine collections had to be processed for shipping to MCLCT within 4 days from receipt at the site laboratories. The 24-h urine samples were tested for oxalate by the oxalate oxidase method [24]. In addition, urinary sulfate, phosphate, citrate, uric acid, sodium, potassium, chloride, calcium, magnesium and creatinine were measured and used to calculate the calcium oxalate supersaturation index by Equil 2 [25]. Plasma oxalate was determined by oxalate oxidase assay [26]. Detection and genotyping of the Oxalobacter strain in stool samples was performed by a polymerase chain reaction-based method using the primers designed from the sequences of the *oxc* (oxalyl-CoA decarboxylase) gene [16].

Table 2. Urinary oxalate excretion expressed as mmol/1.73m^2/day or as molar creatinine ratio in mmol/mol for the *ad hoc* analysis of $n = 37$; P = not significant, baseline versus all weeks

	Baseline	Week 4	Week 8	Week 12	Week 18	Week 24
Oxabact-treated group, $N = 17$						
Mean urinary oxalate mmol/1.73m^2/day (SD)	1.78 (0.6)	1.81 (0.8)	1.66 (0.66)	1.66 (0.57)	1.43 (0.53)	1.45 (0.46)
Mean percentage change from baseline		1.2	−6.6	−6.3	−15.3	−16.4
Mean urinary oxalate mmol/mol (SD)	195 (109)	195 (125)	180 (109)	185 (95)	150 (80)	156 (83)
Mean percentage change from baseline		−1.3	−7.4	−0.85	−16.1	−19.1
Placebo-treated group, $N = 20$						
Mean urinary oxalate mmol/1.73m^2/day (SD)	1.76 (0.64)	1.57 (0.47)	1.83 (0.53)	1.60 (0.55)	1.77 (0.53)	1.54 (0.58)
Mean percentage change from Baseline		−6.6	7.3	−5	7.0	−13.5
Mean urinary oxalate mmol/mol (SD)	178 (70)	158 (58)	177 (62)	164 (65)	175 (73)	169 (83)
Mean percentage change from baseline		−8.9	4.3	−6.5	0.55	−8.1

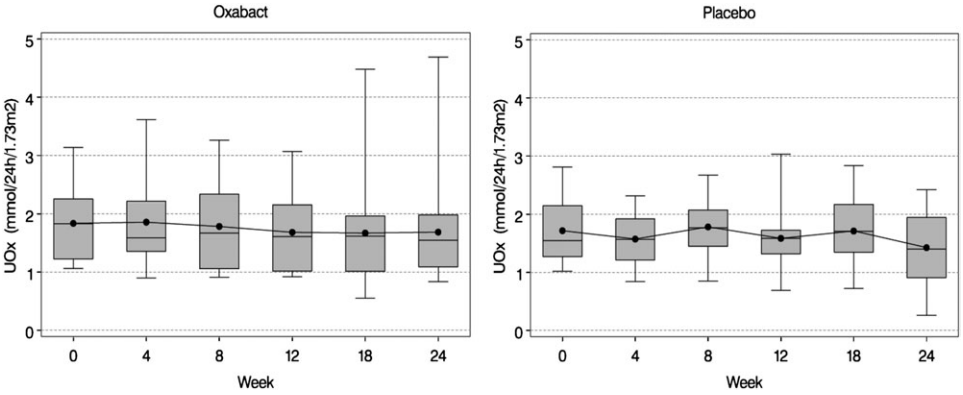

Fig. 1. In the entire study population of 42 patients, mean percent change in urinary oxalate (mmol/1.73m2/day) from baseline to Week 24 was −13% (SD = 25%) in the Oxabact group and −17% (SD = 27%) in the placebo group resulting in a difference between treatment groups of −4% (95% CI: −12−20%) with a P-value of 0.616.

eGFR was calculated using the Schwarz formula in children and the Modification for Diet in Renal Disease (MDRD) study equation for adults [27, 28]. The eGFR were calculated at Weeks 0, 12 and 24 of study. Renal imaging by renal ultrasound was done at all sites at baseline and Week 24 to determine the presence of calcification or stones.

The study was approved by all local Institutional review boards and subjects were considered enrolled after signing the Informed Consent Form and assignment of a unique subject number. Randomization to a treatment number was performed by the unblinded pharmacist at the EU or US drug depot, according to subject location.

Statistical methods

The study was powered to see a 30% difference between the groups. The sample size estimation was based on the results from the pilot study [23], where the standard deviation of the percent change from baseline to Week 4 was 20–25% and the mean percentage change was 50–60%. By assuming a standard deviation of 25%, a 5% level of significance and a sample size of 30, the power was 90% to detect a difference of 30% points between the mean percentage changes in Oxabact versus placebo.

Since the distribution of percent changes in urinary oxalate could be approximated by a normal distribution the parametric Student's *t*-tests and Fischer's exact tests were used instead of nonparametric Wilcoxon rank-sum tests when comparing changes between treatment groups. P-values <0.05 were considered significant.

Results are first shown for all 42 patients who received treatment (12 Cologne, 8 Rochester, 5 Amsterdam, 4 Lyon, 4 + 1 Paris, 4 Birmingham, 2 Hamburg and 1 London). In four patients, the local laboratory did not follow the study procedure for processing of 24-h urine samples. No hydrochloric acid or toluene was added to the aliquots. The aliquots were shipped frozen to MCLCT laboratories to preserve sample stability. Since oxalate measured in the urine samples could have been influenced by this protocol deviation, study results are reported both with and without these four patients. In addition, *ad hoc* analyses were performed in: (i) 37 patients who were compliant with >80% of medication, and in whom urine handling followed study procedure and (ii) patients with urine oxalate above ($n = 18$) or below the median excretion rate of 160 mmol/mol creatinine.

Results

Primary analysis

There was no overall treatment difference between the Oxabact and placebo treatment groups. At baseline, the urinary oxalate (mmol/1.73m^2/day) was similar in the Oxabact (1.84; SD = 0.63) and in the placebo (1.72; SD = 0.55) treatment group (Figure 1). The mean percent change in urinary oxalate (mmol/1.73m^2/day) from baseline to Week 24 was −13% (SD = 25%) in the Oxabact group and −17% (SD = 27%) in the placebo group resulting in a difference between treatment groups of −4% [95% confidence interval (CI): −12 to 20%] with a P-value of 0.616. There was a reduction in urinary oxalate levels in each group but Oxabact had no significant effect when compared to placebo at the primary endpoint (Figure 1). Results were similar using urinary oxalate to creatinine ratio (mmol/mol) instead of urinary oxalate excretion rates. Here, the mean percentage change in the Oxabact treatment group was −14% (SD = 27%) at Week 24 compared with −14% (SD = 26%) in the placebo group; mean treatment difference between groups was 0.7% (95% CI: −16 to 17; P = 0.930).

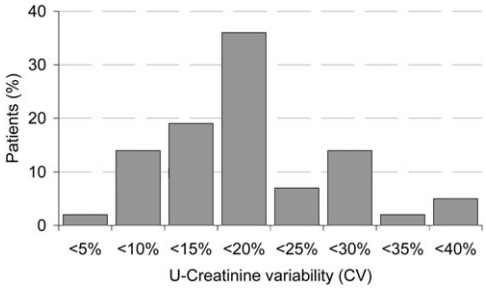

Fig. 2. Variability of urinary creatinine excretion: 28% of patients had abnormal variability >20%.

The number of responders (defined as a 20% or greater reduction of urinary oxalate in mmol/1.73m^2/day from baseline to Week 24) were not significantly different between groups, with 32% of Oxabact and 39% of placebo, respectively, showing a reduction of >20% in urine oxalate, P = 0.750.

The urinary creatinine (U-Cr) excretion within a subject across collections and visits during the study showed a high variability (Figure 2) with 28% of patients having a variability of >20%. There was also a between-center variability with regard to U-Cr excretion values and adolescents, males in particular had a higher variability.

Baseline values for plasma oxalate were 6.3 μmol/L (SD = 3.4) and 8.2 μmol/L (SD = 6.2) in the Oxabact and placebo groups, respectively. There were no

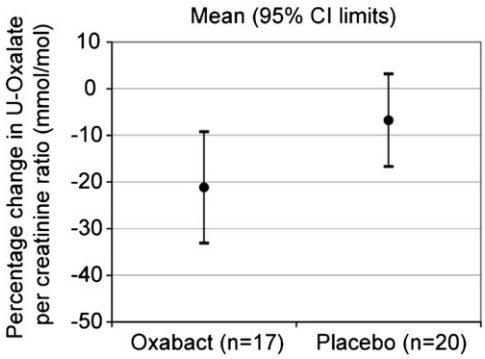

Fig. 3. Percent change in urinary oxalate to creatinine ratio (mmol/mol) by treatment group in the 37 patients who were compliant with >80% of study medication and whose urine collection procedures conformed to study protocol: −21% (SD = 23%) in subjects given Oxabact and −7% (SD = 21%) in placebo (P = 0.056).

Table 3. Percent change in urinary oxalate excretion expressed as molar creatinine ratio (Ox:Cr) in patients with baseline Ox:Cr ratios <160 mmol/mol compared to those >160 mmol/mol

Ox:Cr < 160 mmol/mol, $n = 19$				Ox:Cr > 160 mmol/mol, $n = 18$			
	Mean	95% CI Limits			Mean	95% CI Limits	
Oxabact ($n = 10$)	−17	−34	1	Oxabact ($n = 7$	−24	−47	−9
Placebo ($n = 9$)	−8	−27	11	Placebo ($n = 11$)	−6	−19	7
Oxabact versus Placebo	−9	−33	16	Oxabact versus Placebo	−18	−42	−2
P-value	0.4608			P-value	0.082		

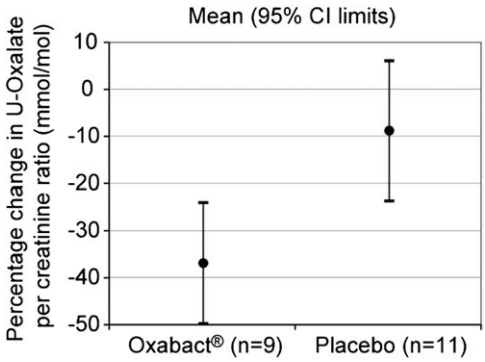

Fig. 4. Percentage change in urinary oxalate to creatinine ratio (mmol/mol) by treatment group in patients enrolled at two major sites ($n = 20$); −37% (SD = 17%) in subjects given Oxabact® and −9% (SD = 22%) in placebo (P = 0.006).

differences in plasma oxalate between the groups in response to treatment. The mean change from baseline to Week 24 was 0.0 μmol/L (SD = 2.3) in the Oxabact and 1.7 μmol/L (SD = 5.9) in the placebo group.

Stratification by GFR, PH type, age or geographical region (EU and USA) did not show any patterns between the groups. There were also no changes seen in the reduction of the urinary calcium oxalate supersaturation. Neither eGFR nor quality of life showed any different patterns between the treatment groups. These endpoints need longer follow-up periods and will be further evaluated in an open-label study.

A sub-study to determine stool *O. formigenes* detection was conducted in 13 subjects enrolled at two clinical sites in Germany. Six of these had received Oxabact treatment. Stool samples at two time points, at baseline (pretreatment) and at end of treatment were available only from six subjects in the study. Two subjects were on placebo and four were on active treatment. The samples that were positive were also subjected to genotyping to classify the strain into Group I or Group II. The OC3 strain HC 1 is a Group I strain. At Week 24, strain HC1 could be detected in the fecal samples from two to four examined subjects on active treatment and in none of those on placebo.

Ad hoc analyses

In the 37 patients compliant with medication and in whom urine processing met study protocol, the change in urinary oxalate excretion rate (mmol/1.73m^2/day) was −19% (SD = 23%) in subjects given Oxabact and −10% (SD = 26%) in placebo, (P = 0.288, Table 2). The change in urinary oxalate to creatinine ratio (mmol/mol) was −21% (SD = 23%) in subjects given Oxabact and −7% (SD = 21%) in placebo (P = 0.06, Figure 3).

The overall proportion of responders (defined as 20% or greater reduction in urinary oxalate to creatinine ratio) following treatment with Oxabact and placebo was 53% (9/17) and 30% (6/20), respectively (P = 0.198). The change in oxalate to creatinine ratio (mmol/mol) in patients with a baseline value above median (>160 mmol/mol) was −24% (SD = 23%) and −6% (SD = 19%) in Oxabact- and placebo-treated groups, respectively, corresponding to a difference of −18% (P = 0.082, Table 3).

Additional analysis of data from the two sites accounting for the largest numbers of study subjects ($n = 20$) resulted in percentage change in oxalate/creatinine ratio of −37% in subjects treated with Oxabact compared with change of −9% in placebo-treated subjects (P < 0.006, Figure 4). Variation of urinary creatinine excretion in these two centers was <15%.

Safety

Oxabact was safe and well tolerated with an adverse event profile similar to placebo. Eighteen subjects (95%) in the Oxabact treatment group and 21 subjects (91%) in the placebo treatment group experienced a total of 148 treatment-emergent adverse events. The most common were gastrointestinal symptoms [14 (74%) and 17 (74%) subjects in the Oxabact and placebo treatment groups, respectively] and infections [11 (58%) and 11 (48%), respectively]. Vomiting was reported in the Oxabact group [8 (42%)] and nausea [7 (30%)], upper abdominal pain [6 (26%)] and diarrhea [6 (26%)] in the placebo group are to be mentioned specifically.

Other common treatment-emergent adverse events were headache [Oxabact: 9 (47%), placebo 10 (43%) subjects] and nasopharyngitis [6 (26%)] in the placebo group. The

majority of adverse events was mild and did not appear related to the study medication; there were no major or definite medication-related adverse events reported in either treatment group.

One subject in each treatment group experienced a serious adverse event. In the Oxabact treatment group, one (5%) subject experienced bronchopneumonia and one (4%) subject in the placebo group was hospitalized with *Escherichia coli*-induced pyelonephritis. Both events were considered unrelated to treatment. In addition, a 12-year-old male passed a kidney stone, which was described as a serious adverse event (SAE). However, this SAE occurred after randomization but prior to receiving study medication. No other patient experienced stone passage, stone growth or new stone formation nor worsening of nephrocalcinosis when comparing baseline with Week 24 ultrasound results. No subjects were withdrawn due to adverse events.

Overall, there were no safety issues raised and the reported AE were equally distributed between the Oxabact and placebo treatment groups in terms of number, severity, relationship and seriousness. Also, no specific safety issue was recognized during the 6-month open-label study.

Discussion

Data from this double-blind placebo-controlled study did not show a treatment effect on reduction of urinary oxalate with Oxabact when compared with placebo. *Ad hoc* analyses in the 37 subjects who were compliant with >80% of the study medication and in whom urine handling procedures followed study protocol did not show an effect by urine oxalate excretion rate per 24 h ($P = 0.29$), but the results suggested an effect when urine oxalate was analyzed by oxalate to creatinine ratio ($P = 0.06$). Since this study included predominantly pediatric patients, strict compliance with complete 24-h urine collections was a particular challenge. Thus, the oxalate/creatinine analysis may be a better indicator of an effect. In addition, subgroup analyses suggested that those with a higher baseline Uox had a greater reduction in urinary oxylate with Oxabact than with placebo, although the difference did not reach statistical significance ($P = 0.082$). Also, the additional analysis of the two largest sites may provide evidence about the possible effects of Oxabact treatment. No safety issues were raised and adverse events were equally distributed between Oxabact and placebo.

The efficacy of *O. formigenes* for reducing urinary oxalate excretion was not demonstrated by this clinical trial, despite favorable results in a previous pilot study [23], and data showing a striking response to *Oxalobacter* or oxalate-degrading enzyme treatment, respectively, in animal models of primary hyperoxaluria [19, 21, 22]. Differences in results could be explained by problems with bioavailability of the *Oxalobacter* supplement used in this study, methodological issues concerning urine collections or other aspects of the current study or biologic ineffectiveness of the agent in humans.

Urine collection, processing and storage may have affected study results. Accurate measurement of urinary oxalate excretion, the primary efficacy outcome measure in this trial, requires strict adherence to urine collection and handling methods. Collection is a critical requirement particularly when results are based on the total 24-h oxalate excretion (mmol/1.73m^2/day) versus a ratio to creatinine excretion (mmol/mol). The former was selected as the primary outcome because of known diurnal variability in urine oxalate excretion [29]. As the urine creatinine excretion results in this study show a larger than expected within-subject variability (up to >40% between consecutive urine collections, compared with 10–15% expected [30, 31], and as this serves as an indicator for completeness of 24-h urine collections, inadequate urine collection appeared to be a major obstacle to accurate measurement of the primary endpoint in this study. *Ad hoc* analysis of the outcome by oxalate to creatinine ratios was performed in an effort to reduce the effect of collection variability. This index also suggests an Oxabact effect ($P = 0.06$), though the effect size (21% reduction after 24 weeks in the Oxabact group alone and 14% when compared with placebo) was less than expected, compared with results in the earlier open-label Phase I/II study which demonstrated a 40–60% reduction after 4 weeks of treatment in PH patients with similar baseline urinary oxalate levels [23]. Other factors may have contributed to the reduced effect size between earlier and recently completed studies, including use of a central laboratory requiring longer storage and shipping time for samples. It is difficult to judge the impact this variability in procedures may have had on the analysis results, but it suggests that between sites variability may be important.

The Oxabact product used in the present study had an enteric-coated hydroxypropyl-methylcellulose (HPMC) capsule, different from the enteric-coated gelatin capsule used in the phase 1/2 study [23]. The capsule changes were implemented to improve the shelf life of the product. The change in enteric coating may have reduced the likelihood of enteric delivery or colonization, with dissolution of the coating further down the intestinal tract, or perhaps because dissolution did not occur consistently in all patients. This may be supported by the fact that the Oxabact strain HC 1 was only detected in the fecal samples from two out of four subjects on active treatment examined in a German substudy population.

Primary hyperoxaluria is a rare orphan disease with very small numbers of patients available for clinical studies [7–9]. In such circumstances, issues with study design, execution and patient compliance can significantly affect the overall study results. This double-blind study was the first large-controlled study in PH patients and important data on the disease, patient population, medical management and follow-up from a global perspective were collected. There was greater variability in urine oxalate excretion in individual patients during both baseline and treatment collection periods than expected. Whether this degree of variability is related to characteristics of the disease or to urine collection methods is unclear.

There is evidence that intestinal degradation of endogenous oxalate is of physiologic importance and potential pharmacologic benefit. It was shown recently that either natural or artificial colonization with *O. formigenes* in rats promotes reduction of urinary oxalate excretion [19, 32].

In addition to its intraluminal oxalate-degrading capacity, *Oxalobacter* interacted physiologically with colonic mucosa, stimulating an intestinal oxalate transporter and inducing enteric oxalate excretion. In mice that received oxalate-degrading enzyme, hyperoxaluria was reduced and nephrocalcinosis prevented. This effect was shown in two *agt1* knockout mouse models, one closely resembling primary hyperoxaluria (AGT deficiency) and the other using the *agt1* knockout mice under ethylene glycol feeding, hence an even higher oxalate challenge. Differences in animal and human physiology that might account for such a discrepancy in outcome in our study have not so far been recognized. Thus, these animal data suggest that intestinal oxalate degradation and excretion can indeed be a realistic treatment option in human patients with hyperoxaluria [19, 21, 32].

We conclude that Oxabact treatment is safe and well tolerated. Disappointingly, there was no difference between Oxabact and placebo in reduction of urinary oxalate when data from all study participants were evaluated. Nevertheless, the *ad hoc* analysis of study data and the data from the two largest centers suggests that treatment with Oxabact may lower urinary oxalate in patients with primary hyperoxaluria. Information is now available to improve design and execution of further clinical studies.

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Conflict of interest statement. None declared.

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