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Oxalobacter formigenes: a potential tool for the treatment of primary hyperoxaluria type 1

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Primary hyperoxaluria is characterized by severe urolithiasis, nephrocalcinosis, and early renal failure. As treatment options are scarce, we aimed for a new therapeutic tool using colonic degradation of endogenous oxalate by *Oxalobactor formigenes*. *Oxalobacter* was orally administered for 4 weeks as frozen paste (IxOC-2) or as enteric-coated capsules (IxOC-3). Nine patients (five with normal renal function, one after liver–kidney transplantation, and three with renal failure) completed the IxOC-2 study. Seven patients (six with normal renal function and one after liver–kidney transplantation) completed the IxOC-3 study. Urinary oxalate or plasma oxalate in renal failure was determined at baseline, weekly during treatment and for a 2-week follow-up. The patients who showed >20% reduction both at the end of weeks 3 and 4 were considered as responders. Under IxOC-2, three out of five patients with normal renal function showed a 22–48% reduction of urinary oxalate. In addition, two renal failure patients experienced a significant reduction in plasma oxalate and amelioration of clinical symptoms. Under IxOC-3 treatment, four out of six patients with normal renal function responded with a reduction of urinary oxalate ranging from 38.5 to 92%. Although all subjects under IxOC-2 and 4 patients under IxOC-3 showed detectable levels of *O. formigenes* in stool during treatment, fecal recovery dropped directly at follow up, indicating only transient gastrointestinal-tract colonization. The preliminary data indicate that *O. formigenes* is safe, leads to a significant reduction of either urinary or plasma oxalate, and is a potential new treatment option for primary hyperoxaluria.

Kidney International (2006) **70,** 1305–1311. doi:10.1038/sj.ki.5001707; published online 19 July 2006

KEYWORDS: oxalobacter formigenes; treatment; primary hyperoxaluria

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Received 3 March 2006; revised 24 May 2006; accepted 31 May 2006; published online 19 July 2006

Primary hyperoxaluria type 1 (PH 1) is a rare, autosomal recessive inherited disease caused by low or absent activity of the liver-specific peroxisomal alanine:glyoxylate aminotransferase.[1–3] Elevated urinary oxalate excretion (U_{Ox} > 1 mmol/1.73 m^2 body surface area (BSA)/day, normal <0.5[1,3]) and urinary super saturation with respect to calcium-oxalate (β_{UCaOx} > 10 rel. units, normal male <6.3, normal female <5.5[4]) are the hallmarks of the disease-causing renal calculi, medullary nephrocalcinosis, or both.[1] With disease progression and declining renal function, systemic calcium-oxalate (CaOx) crystal deposition occurs.

Current therapeutic options are scarce and are only completely successful in a minority of patients.[5–7] Hence, we aimed for a new therapeutic tool and speculated that colonic degradation of oxalate by *Oxalobacter formigenes* (*O. formigenes*) may provide a suitable transepithelial gradient for the enteric elimination of endogenously produced oxalate.

O. formigenes is an obligate anaerobic bacterium naturally colonizing the colon of vertebrates, including humans, which utilizes oxalic acid as its sole source of energy.[8] Oxalate degradation by *O. formigenes* involves three unique proteins, an oxalate:formate membrane transporter, oxalyl-CoA decarboxylase, and formyl-CoA transferase. The genes for all three proteins have been cloned and sequenced.[9–11] Robust colonization with *O. formigenes* is in the range of 10^7–10^8 colony forming units (CFUs) per gram wet fecal sample, translating to a degrading capacity of up to 1 g (11.1 mmol) of oxalate/day in the human gut.

Children acquire *O. formigenes* during early childhood and fecal samples from 70 to 80% of the adult population test positive for this bacterium.[12–16] A much lower *O. formigenes* colonization rate was found in recurrent CaOx kidney stone-formers and in subjects with enteric hyperoxaluria.[13,14,17–20] The importance of *O. formigenes* in degrading dietary oxalate has been shown in animal models where significant decreases in U_{Ox} occurred following either colonization with *O. formigenes*[13] or its daily administration.[21]

Besides absorption, oxalate secretory pathways for extra-renal oxalate elimination have also been recently identified in the large intestine.[22] It has been hypothesized that intestinal degradation of oxalate by *O. formigenes* can

contribute to maintenance of a transepithelial gradient favoring a passive paracellular movement of oxalate from blood to the intestinal lumen.[22] Recent work by Hatch with rat colonic mucosa to modulate the handling of oxalate in colonized animals leading to the induction of enteric secretion/excretion. The ability of *O. formigenes* to stimulate secretion of endogenously produced oxalate and to degrade it in the intestine (use as a carbon source) supports the hypothesis that this microorganism can be of benefit for PH patients by lowering their urinary oxalate excretion.

RESULTS

The individual urinary oxalate/creatinine ratios in both studies are shown in Figures 1 and 2 and mean urinary oxalate excretion $\pm$s.d. is given in Tables 1 and 2. In addition, mean values of other lithogenic (calcium) and stone-inhibitory (citrate, magnesium) substances, as well as β_{UCaOx} ($\pm$s.d., range) are presented in Tables 1 and 2. All the subjects showed a good compliance with the 24 h urine collection as was evident from total volumes and urinary creatinine levels (Tables 1 and 2).

In the IxOC-2 (*Oxalobacter* was orally administered for 4 weeks as frozen paste) study, U_{Ox} was monitored in five patients with normal renal function (Figure 1). Three were found to be responders and their urinary oxalate/creatinine ratios showed a 22–48% reduction as compared to the baseline. Mean U_{Ox} levels (expressed as mmol/1.73 m^2/24 h) for all the patients, although lower at weeks 3 and 4, did not decrease significantly during treatment with IxOC-2 (Table 1, P = NS). A significant increase in mean urinary calcium excretion was observed at weeks 4 and 5 ($P<0.02$). Concomitantly, urinary β_{CaOx} increased significantly at week 4 of treatment ($P<0.05$).

In patient no. 10 after successful combined liver–kidney transplantation (LKTx), urinary oxalate was also monitored, but did not drop significantly during treatment. However, her P_{Ox} declined from 18 to 5.5 μmol/l at the end of week 3 (ref. value is 6.3 $\pm$ 1.1 μmol/l), but increased again after termination of treatment.

In the IxOC-3 (*Oxalobacter* was orally administered for 4 weeks as enteric-coated capsules) study, U_{Ox} was monitored in six patients with normal renal function (Figure 2). Four out of the six patients were found to be responders and their urinary oxalate/creatinine ratios showed a 38.5–92% reduction as compared to the baseline values. Patient no. 19, who was treated after successful combined LKTx, again did not show a reduction in urinary oxalate excretion. In this study, a statistical significant decrease in the mean U_{Ox} excretion (mmol/1.73 m^2/24 h) was achieved in all weeks as compared to baseline and the decline was highly significant ($P<0.001$) at the end of treatment weeks 3 and 4 (Table 2). Mean urinary oxalate increased again within 1 week of stopping the treatment, but another decrease was observed at the end of week 6. However, long-term follow-up in the majority of the patients (12 months) indicated that the urinary oxalate excretion returned back to their historical higher levels. Urinary β_{CaOx} decreased significantly at weeks 3 and 4 of IxOC-3 treatment (Table 2, $P<0.05$).

A decrease of plasma oxalate levels was observed in 10/13 patients with normal or stable kidney function leading to a significant reduction of mean plasma oxalate in both studies (Tables 1 and 2, $P<0.05$).

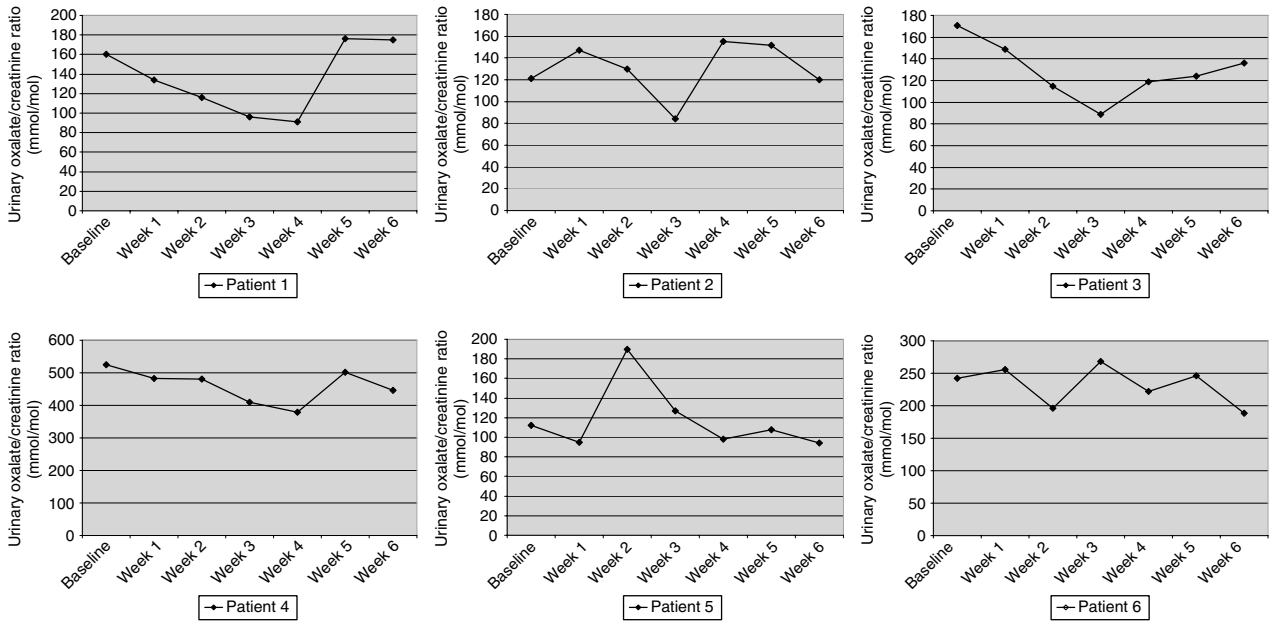

Figure 1 | Urinary oxalate/creatinine ratio (mmol/mol) in patients with normal renal function (patient no. 1–4 and 9) and in one patient after combined liver–kidney transplantation (no. 10) with stable kidney function under treatment with IxOC-2 at baseline, treatment phase (weeks 1–4) and at short follow up (weeks 5 and 6).

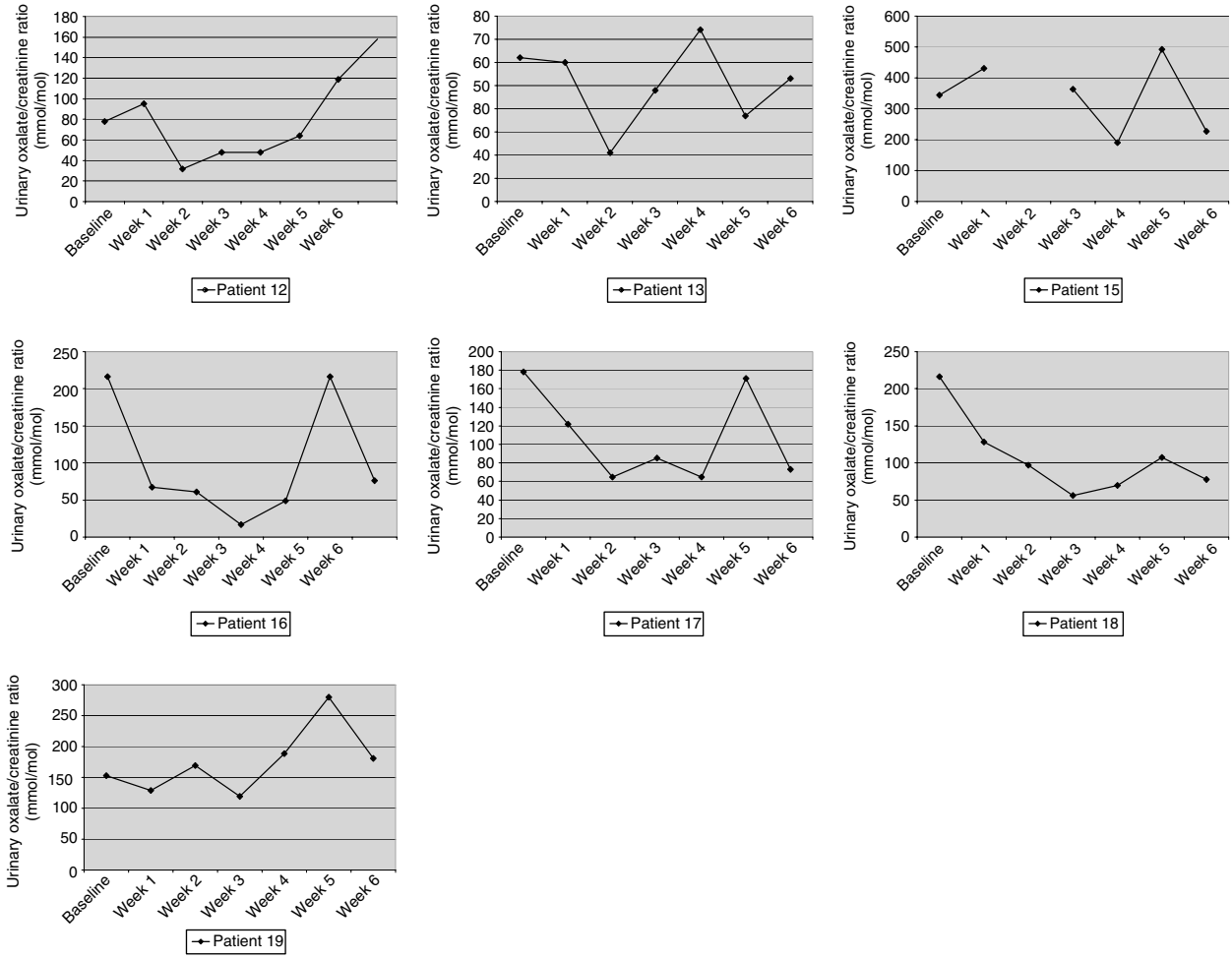

Figure 2 | Urinary oxalate/creatinine ratio (mmol/mol) in patients with normal renal function (no. 12–18) and in one patient after combined liver–kidney transplantation and stable kidney function (no. 19) under treatment with IxOC-3 at baseline, treatment phase (weeks 1–4) and at short follow up (weeks 5 and 6). Week 2 urine of patient no. 15 got lost in mail.

Treatment efficacy was followed by weekly measurement of plasma oxalate in three end stage renal failure (ESRF) patients enrolled in the IxOC-2 study. Plasma oxalate levels decreased under treatment in patients no. 6 and no. 8, reaching nearly normal levels in patient no. 8 at weeks 3–5 (Figure 3). Patient no. 6 was treated for 5 weeks. The plasma oxalate levels remained low in both patients 1 week after treatment was stopped but started to increase by the end of 2-week post-treatment follow-up. Consistent decrease in plasma oxalate was not observed in Patient no. 11 in whom some compliance issues were suspected. A lower P_{Ox} level was only seen in week 1 of treatment in this patient.

Patient no. 10 after combined liver–kidney transplantation and the ESRF patient no. 6, both with systemic oxalosis, reported an amelioration of clinical symptoms under therapy, with less pain due to oxalate osteopathy (no. 6 and 10) or CaOx skin deposits (no. 10).

In the IxOC-2 study, fecal samples were tested for the presence/absence of *O. formigenes* by the polymerase chain reaction (PCR)-based method. Only patient no. 1 was colonized at baseline and remained colonized throughout the study and thereafter. In all other patients, fecal recovery of orally fed *O. formigenes* was observed during the treatment phase, which was lost during the 2 week follow-up except in patient no. 6.

In the IxOC-3 study, two patients were colonized at baseline, including patient no. 16, who already participated in the IxOC-2 study. Fecal recovery of orally fed *O. formigenes* was highly variable in this study, and at the end of the 4-week treatment only four out of seven patients showed the bacterium in their stool specimens. Only patients who were colonized at baseline remained colonized 2-weeks post-treatment.

The intestinal oxalate absorption was $\leqslant 10\%$ in all the patients except for patient no. 19, whose baseline oxalate absorption was 11%. All values were within or even below $7.9 \pm 4\%$ (mean $\pm$ s.d. of the reference range[24]). In the IxOC-2 study, a significant difference in the mean oxalate absorption at baseline and post-treatment was observed ($P < 0.02$, Table 1). However, no difference was found in the

Table 1 | Mean values ± s.d. (range) for urinary excretion parameters, urinary calcium-oxalate saturation, plasma oxalate, and intestinal oxalate absorption of the patients with normal (No. 1–4 and No. 9) or stable (No. 10) renal function included in the IxOC-2 study

	Baseline, Mean ± s.d. (range)	Week 1 Mean ± s.d. (range)	Week 2 Mean ± s.d. (range)	Week 3 Mean ± s.d. (range)	Week 4 Mean ± s.d. (range)	Week 5 Mean ± s.d. (range)	Week 6 Mean ± s.d. (range)
Urinary oxalate (mmol/1.73m^2/24 h)	1.64 ± 1.17 (0.64–3.82)	1.96 ± 1.68 (0.71–5.24)	1.93 ± 1.27 (0.88–4.38)	1.42 ± 0.86 (0.77–2.87)	1.53 ± 0.92 (0.81–3.18)	1.39 ± 0.42 (1.05–1.96)	1.37 ± 0.55 (0.81–2.32)
Calcium (mg/kg body weight/24 h)	1.5 ± 0.73 (0.6–2.35)	1.64 ± 0.25 (1.37–2.04)	1.51 ± 0.52 (1.05–2.17)	1.69 ± 0.42 (1.14–2.13)	2.07 ± 0.77[a] (1.02–2.99)	2.21 ± 0.58[b] (1.55–2.95)	1.74 ± 0.87 (0.96–3.2)
Citrate (mmol/1.73m^2/24 h)	3.7 ± 2.11 (1.09–6.11)	3.43 ± 0.8 (2.37–4.56)	3.2 ± 1.72 (1.16–5.67)	3.64 ± 1.34 (2.28–5.02)	3.59 ± 1.85 (1.17–5.5)	2.97 ± 0.82 (1.86–3.8)	3.42 ± 1.78 (2.4–6.99)
Magnesium (mmol/1.73m^2/24 h)	4.58 ± 1.78 (2.13–7.66)	6.1 ± 2.41 (3.66–10.23)	6.06 ± 1.53[a] (4.36–7.84)	5.9 ± 1.4 (3.58–7.96)	6.73 ± 3.06 (3.29–11.69)	6.88 ± 1.58[c] (4.34–9.05)	5.65 ± 2.0 (3.38–8.04)
β_{UCaOx} (rel. units)	4.44 ± 1.22 (2.94–6.46)	4.57 ± 1.21 (3.45-6.39	4.88 ± 0.72 (3.84–5.8)	4.51 ± 0.7 (3.3–5.4)	5.49 ± 1.6[c] (3.07–7.71)	6.89 ± 2.95 (3.25–12.11)	4.52 ± 1.75 (2.49–7.27)
Urine creatinine (Mmol/24 h)	4.9 ± 2.14 (2.73–7.48)	5.15 ± 1.38 (3.59–7.17)	6.38 ± 1.98[b] (4.05–9.39)	5.73 ± 1.66 (3.34–7.2)	5.98 ± 1.72 (3.75–8.41)	5.13 ± 1.9 (1.72–7.25)	5.95 ± 2.08[b] (3.9–8.06)
Urine volume (l)	2.22 ± 0.98 (0.5–3.2)	2.05 ± 1.22 (0.5–4.05)	2.02 ± 0.95[c] (0.55–3.1)	1.95 ± 1.02 (0.45–3.6)	2.0 ± 0.9 (0.8–3.7)	2.44 ± 1.24 (0.7–3.7)	1.89 ± 0.96 (0.65–3.52)
Plasma oxalate (μmol/l)	10.17 ± 5.52 (2.67–18.23)	—	—	—	—	6.77 ± 3.73[c] (0.64–11.76)	—
Oxalate absorption (%)	8.9 ± 1.99 (5.8–11.4)	—	—	—	—	6.4 ± 2.77[a] (1.6–8.9)	—

[a]$P < 0.02$ vs baseline, [b]$P < 0.002$ vs baseline and [c]$P < 0.05$ vs baseline.

Table 2 | Mean values ± s.d. (range) for urinary excretion parameters, urinary calcium-oxalate saturation, plasma oxalate, and intestinal oxalate absorption of the patients with normal (No. 12 and No. 15–17) or stable (No. 13, No. 18 and No. 19) renal function included in the IxOC-3 study

	Baseline Mean ± s.d. (range)	Week 1 Mean ± s.d. (range)	Week 2 Mean ± s.d. (range)	Week 3 Mean ± s.d. (range)	Week 4 Mean ± s.d. (range)	Week 5 Mean ± s.d. (range)	Week 6 Mean ± s.d. (range)
Urinary oxalate (mmol/1.73m^2/24 h)	1.85 ± 0.49 (0.74–2.49)	0.83 ± 0.44[a] (0.23–1.8)	1.04 ± 0.42[a] (0.34–1.5)	0.53 ± 0.18[b] (0.14–0.75)	0.74 ± 0.23[b] (0.23–0.94)	1.59 ± 0.71 (0.37–2.5)	0.85 ± 0.36[a] (0.48–1.64)
Calcium (mg/kg body weight/24 h)	0.93 ± 0.49 (0.42–1.89)	0.71 ± 0.4 (0.34–1.39)	1.07 ± 1.22 (0.12–3.42)	0.72 ± 0.47 (0.18–1.51)	1.27 ± 0.52 (0.59–2.07)	1.64 ± 1.1 (0.81–4.08)	1.37 ± 0.96 (0.66–3.42)
Citrate (mmol/1.73m^2/24 h)	1.67 ± 0.9 (0.53–2.79)	1.45 ± 0.91 (0.35–2.89)	1.66 ± 0.94 (0.5–2.82)	1.4 ± 0.77 (0.39–2.28)	1.78 ± 1.13 (0.54–3.34)	2.45 ± 1.71 (0.53–5.37)	1.8 ± 0.78 (0.73–2.81)
Magnesium (mmol/1.73m^2/24 h)	4.16 ± 2.08 (1.08–6.65)	4.26 ± 2.36 (2.39–7.94)	5.49 ± 1.5 (4.01–7.49)	5.67 ± 1.89 (3.67–8.43)	6.21 ± 2.25 (3.0–8.25)	5.06 ± 2.71 (2.74–10.08)	5.16 ± 1.92 (3.14–8.63)
β_{UCaOx} (rel. units)	4.83 ± 2.45 (1.08–8.14)	2.91 ± 2.39 (1.16–2.39)	3.31 ± 3.1 (1.25–9.06)	2.62 ± 1.57[c] (0.6–4.79)	3.06 ± 1.3[c] (1.58–5.43)	7.35 ± 3.43 (1.11–11.81)	2.89 ± 1.15 (1.16–4.01)
Urine creatinine (Mmol/24 h)	9.06 ± 3.93 (3.47–14.77)	6.67 ± 3.58[a] (3.26–10.65)	12.26 ± 3.23[c] (8.4–17.75)	9.41 ± 5.94 (8.83–21.7)	10.07 ± 4.88 (3.1–17.44)	8.62 ± 3.68 (2.47–13.75)	8.43 ± 4.06 (2.65–13.31)
Urine volume (l)	2.4 ± 0.91 (1.55–4.2)	2.55 ± 0.55 (1.95–3.55)	2.51 ± 0.87 (1.8–4.05)	2.39 ± 0.84 (1.2–3.8)	2.59 ± 0.81 (1.8–4.1)	2.71 ± 0.89[c] (1.65–4.28)	2.54 ± 0.88 (1.9–4.2)
Plasma oxalate (μmol/l)	12.32 ± 4.98 (8.31–20.24)	—	—	—	—	8.06 ± 3.34[c] (1.69–11.34)	—
Oxalate absorption (%)	6.43 ± 3.91 (1.6–11.4)	—	—	—	—	5.13 ± 2.59 (2–8.3)	—

[a]$P < 0.002$ vs baseline, [b]$P < 0.001$ vs baseline and [c]$P < 0.05$ vs baseline.

Table 3 | Clinical and laboratory data of the patients who completed the IxOC-2 and IxOC-3 study

Subject number	Sex	Age (years)	Diagnosis	S-Crea (mg/dl)	AGXT mutation	AGT (μmol/h/mg protein)	Renal ultrasound	Medication
1	F	14	PH 1	1.0	508G>A (hz)	5.02	Nephrocalcinosis	B6, Alkali-citrate
2/16	M	10/11	PH 1	0.7	508G>A (hz)	5	Urolithiasis	B6, Alkali-citrate
3/17	M	8/9	PH 1	0.6	508G>A (hz)	ND*	Urolithiasis	B6, Alkali-citrate
4/15	F	5/6	PH 1	0.4	33delc (hoz)	1.7	Nephrocalcinosis	B6, Alkali-citrate
6/14	F	21/22	PH 1	ESRF	508G>A (hoz)	9.4	Nephrocalcinosis	B6
8	M	3.5	PH 1	ESRF	508G>A (hz)	5.3	Nephrocalcinosis	B6
9	M	8	PH 1	0.53	n.m.	4.2	Urolithiasis	B6, Alkali citrate
10/19	F	49/50	PH 1	1.6	508G>A (hz)	6.2	Urolithiasis Nephrocalcinosis Syst. oxalosis	B6, Alkali citrate Steroids, CyA MMF
11	M	8	PH 1	ESRF	ND	ND	Urolithiasis	No PH medicines
12	M	15	PH 1	0.91	508G>A (hz)	5.5	Urolithiasis	B6, Alkali-citrate
13	M	12.5	PH 1	1.6	508G>A (hoz)	9.0	Nephrocalcinosis	B6, Alkali-citrate
18	M	15.5	PH 1	1.56	508G>A (hz)	5.3	Urolithiasis	B6, Alkali citrate

AGT, alanine:glyoxylate aminotransferase (normal=19.1–47.9 μmol/h/mg protein); ESRF, end-stage renal failure; F, female; hz, heterozygote; hoz, homozygote; M, male; MMF, mycophenolate mofetil; ND, not done; nd*, not done, as siblings value was informative; n.m., no mutation yet found; PH 1, primary hyperoxaluria type 1.
Patients no. 2-4, 6, and 10 participated in both studies.

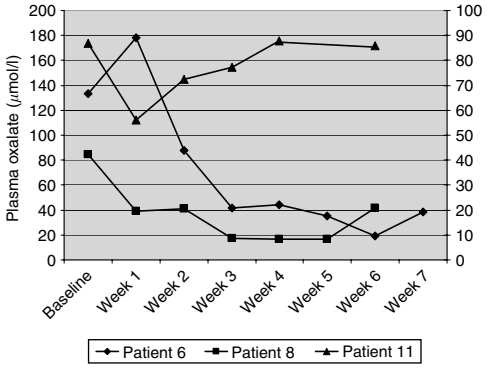

Figure 3 | Plasma oxalate levels in three patients with ESRF treated with IxOC-2 for 4 or 5 weeks (no. 6), respectively. Right, *Y*-axis depicts patient no. 8. Week 5 blood sample in patient no. 11 was only drawn 30 min after the start of dialysis and hence, plasma oxalate was not determined.

IxOC-3 study (Table 2). The percentage of $[^{13}C_2]$oxalate absorbed remained unchanged in two subjects, decreased in nine subjects, and slightly increased in two subjects.

Overall, administration of *O. formigenes* was found to be a safe treatment. Results of complete blood chemistry and hematology performed at baseline and at week-5 showed no significant changes in any of the parameters (data not shown). We did not see any major side effect of oral *O. formigenes* administration. Patients were only complaining about the smell of the preparation (IxOC-2) and flatulence was infrequently reported in both studies.

DISCUSSION

We were able to show that oral *O. formigenes* administration is safe, that it transiently colonizes the intestinal tract, and that it exerts its metabolic activity to enhance the non-urinary removal of endogenous oxalate through enteric elimination, thus supporting previous data in animal models.[22,23] The enteric elimination of oxalate has been shown to be both through passive movements across a transepithelial gradient or through specific colonic oxalate secretory pathways.[22] Enteric elimination is expected to be predominant in PH patients, as their P_{Ox} levels are at the higher end of normal or even elevated. This is supported by our observation of a significant reduction of P_{Ox} in the patients with normal or stable renal function in both studies as well as in the two ESRF patients with good compliance in the IxOC-2 study.

The IxOC-3 formulation, delivering *O. formigenes* safely past the acidic conditions of the stomach, produced a >35% decrease of urinary oxalate excretion (mmol/mol creatinine) in 4/6 patients with normal renal function at treatment weeks 3 and 4. Reduction in the mean urinary oxalate excretion at weeks 3 and 4 of treatment concomitantly produced a significant decrease in the urinary CaOx-saturation index at these weeks. The only available treatment for reduction of urinary oxalate in PH 1 is the pyridoxine therapy and considering the proposed mode of action of pyridoxine, this treatment is not effective in patients with PH type 2. Pyridoxine administration in daily doses of 5–20 mg/kg body weight per day has been found to be effective at lowering the urinary excretion of oxalate in approximately 30% of patients (6). Also, a 30% or greater decrease of urinary oxalate upon pyridoxine administration is considered a clinically relevant value (1).

Every little decrease in U_{Ox} or P_{Ox} produces a corresponding decline in the CaOx saturation levels in urine or plasma, thereby reducing the risk of recurrent urolithiasis, progressive nephrocalcinosis, or systemic oxalosis. Reducing the U_{Ox} levels in the approximate range of our short-term results during long-term treatment would hence dramatically lessen the risk of kidney damage and concomitant renal failure in PH patients.

No kind of renal replacement therapy is capable of eliminating sufficient amounts of oxalate.[25,26] Hence, systemic oxalosis develops, the course of the disease deteriorates,

and later lessens the success of combined LKTx. Taken into consideration, that even forced hemodialysis, for example, five times per week, and additional peritoneal dialysis does not sufficiently reduce P_{Ox} over time, our results are of utmost importance for the PH patients on dialysis.[27] Although we did not achieve a decline of P_{Ox} in all three ESRF patients, two arguments still support the thesis that oxalate is sufficiently eliminated via the intestinal tract in ESRF: (1) we achieved a significant reduction in P_{Ox} in the two patients with good compliance (according to the diary, *O. formigenes* was not administered every day in patient 11 since study week 2), and (2) clinical signs of systemic oxalosis ameliorated in patients 6 (ESRF) and patient 10 (post combined LKTx).

Although *O. formigenes* was detected in fecal samples in the majority of patients during the treatment phase, it was not correlated with the extent of treatment efficacy. The culture and PCR-based detection of *O. formigenes* in the stool samples is a qualitative test indicative of only the presence/absence of the bacterium and therefore in this study a correlation between the level of colonization and the reduction in urinary oxalate excretion could not be measured. Detection and quantification of bacteria in stool samples has been reported to have low sensitivity and specificity due to the nature of the sample matrix. Studies with probiotic strains of *Lactobacilli* have shown that fecal recovery of an orally fed strain starts with the consumption of at least a 10^9 CFUs/day dose of encapsulated *lactobacilli*.[28] Similarly, a double-blind, placebo-controlled study using encapsulated *L. fermentum* ME-3 at a daily dose of log 9.2 CFUs showed that although the ME-3 strain did not reach detectable levels in fecal samples, it exerted its metabolic activity in the gut and produced a measurable biologic effect.[29] In the present study, although the fecal recovery of *O. formigenes* was found to be higher with IxOC-2 treatment as compared with IxOC-3, due to higher numbers of cell counts fed, it is believed that the enteric-coated IxOC-3 capsules delivered metabolically active live *O. formigenes* to the colonic segment of the gastrointestinal-tract, which is the main site for enteric elimination pathways.[22] As observed with *O. formigenes* treatment, many studies with bacterial supplementation have shown that the ingested bacterial strains, which survive the passage through the gut, transiently exert their biological effect but neither persist nor become a part of the normal gut microflora.[29,30]

The oxalate absorption test remained normal $<10\%$ or in the wider normal range found for healthy adults of 2.2–18.5% in all patients.[24] This result appears reasonable since about 80% of the oxalate absorption takes place in the upper intestine, normally not colonized by bacteria. The fact that the excretion of orally supplied oxalate is unaffected clearly demonstrates that it is the endogenous oxalate that is removed by *O. formigenes*.

As treatment options in patients with PH are limited, new therapeutic regimens are clearly needed. The data obtained in this study let us speculate that *O. formigenes* might be a very promising new kind of treatment for this mostly devastating disease. It seems possible that endogenously produced oxalate can be removed via the intestinal tract in addition to being excreted via the kidneys. A decrease in U_{Ox} will reduce β_{UCaOx} and hence the risk of recurrent urolithiasis or progressive nephrocalcinosis. According to the results obtained in these two clinical studies, treatment with *O. formigenes* may prove to be a viable treatment option for PH patients with normal kidney function, with ESRF and severe post-transplant oxalosis. However, further multicenter studies with a larger patient population are clearly needed.

MATERIALS AND METHODS

Our study was divided into two parts according to the mode of delivery of *O. formigenes*, to be given b.i.d. together with the main meals for 4 weeks. In the first study, *O. formigenes* was administered as a frozen cell paste containing 1 g live cells equivalent to $>10^{10}$ CFUs (IxOC-2 study). In the second study, the patients took two enteric-coated capsules per dosing (IxOC-3 study). Each capsule contained 137 mg of lyophilized bulk powder of freeze-dried live cells equivalent to $\sim 10^7$ CFUs. The latter allowing a safe delivery of the preparation past the stomach acid. Both preparations were provided by Ixion Biotechnology Inc., Alachua, FL, USA. During both studies, the dosing compliance, adverse events, and concomitant medication were monitored through the patient's diary.

PH 1 patients were eligible for the study and their clinical data is shown in Table 3. Of the 11 patients included in the IxOC-2 study (patients no. 1–11), nine completed the study, five with normal renal function, one after combined LKTx and severe systemic oxalosis, and three patients with ESRF and hemodialysis treatment. One patient (no. 5) turned out to be not eligible, as she was finally diagnosed with PH 2. The other patient (no. 7) was excluded after the baseline examinations were performed, as the parents declined their consent.

In the IxOC-3 study, eight patients were included (Table 3, patient no. 12–19) and seven patients completed the study, six patients with normal or stable renal function and one patient after LKTx and stable graft function. Owing to noncompliance, patient 14 with ESRF was removed from the IxOC-3 study. This patient eventually received an LKTx. Four patients had also completed the treatment in the IxOC-2 study. Patient 15, a five-year-old girl, was only able to swallow one capsule IxOC-3 per dosing.

Before treatment with *O. formigenes*, baseline evaluation included 24 h urine analysis, a $[^{13}C_2]$oxalate absorption test, a plasma oxalate (P_{Ox}) determination, and stool analysis for *O. formigenes*. Safety of the treatment was followed by performing standard clinical laboratory tests (e.g., complete blood chemistry, hematology, and urine analysis) before and after the treatment. A β-HCG analysis was performed to exclude pregnant women from the study.

During the administration of *O. formigenes*, the 24 h urine or plasma samples (in the ESRF patients) were collected and analyzed weekly. Oxalate absorption and P_{Ox} were again analyzed at week 5 in patients with normal renal function. During treatment, subjects took their normal medication, ate their normal western, but low oxalate diet, and patients with normal kidney function kept their fluid intake >1.5 l per m^2 body surface area. Weeks 5 and 6 of the study served as short-term follow-up. Patients are since longitudinally followed in our outpatients clinics.

For urine collection, thymol '5% in isopropanol' was added for preservation. Total urine volume and pH was recorded and urinary oxalate, sulfate, phosphate and citrate, urinary uric acid, sodium, potassium, chloride, calcium, magnesium, specific gravity, and creatinine were measured and β_{UCaOx} was calculated according to previous references.[4,31] Urinary oxalate was expressed as mean 24 h urinary excretion $\pm$s.d. in mmol/1.73 m^2/24 h, but as a molar oxalate/creatinine ratio (mmol/mol) for individual patient data. P_{Ox} analysis was performed via ion chromatography.[32]

In the IxOC-2 study, detection of *O. formigenes* in stool samples was performed using the PCR-based test.[17] In the IxOC-3 study, *O. formigenes* detection was performed by culture at the clinical site as well as by PCR.[17]

For the oxalate absorption test, [$^{13}C_2$]-oxalate was used and the absorption was measured by a gas chromatography-mass spectrometry method as previously published.[24]

A $>$20% reduction of urinary oxalate under treatment was determined as successful end point in patients with normal renal function and patients with such a reduction at both weeks 3 and 4 of treatment were considered to be responders. For each follow-up visit, the differences in the mean U_{Ox} for that visit and the mean baseline U_{Ox} were tested for a difference from zero using the paired *t*-test (SAS version 9.1 software, SAS, Cary, NC, USA) and $P<0.05$ were judged significant. The study was approved by the Ethical Committees of the faculties of medicine of the Universities of Bonn and Cologne, especially with regard to the use of the labeled oxalate. Informed consent was obtained from the patients or their parents for plasma and stool analysis, for the oxalate absorption test, and for the application of either the IxOC-2 or IxOC-3 *O. formigenes* preparation.

ACKNOWLEDGMENTS

This work was supported in part by grants of the Deutsche Forschungsgemeinschaft (Un 91/3 and He 1132/11-4) and from Ixion Biotechnology Inc. (grant No. Ho), Alachua, FL, USA. Harmeet Sidhu and Poonam Kaul previously worked at Ixion Biotechnology Inc., which provided the study preparations IxOC-2 and IxOC-3. This work was presented in part as an oral presentation at the 37th Annual Meeting of the Amercian Society of Nephrology, St Louis, MS, USA.

REFERENCES

1. Leumann E, Hoppe B. The primary hyperoxalurias. *J Am Soc Nephrol* 2001; **12**: 1986–1993.
2. Danpure CJ, Jennings PR, Watts RWE. Enzymological diagnosis of primary hyperoxaluria type I by measurement of hepatic alanine:glyoxylate aminotransferase. *Lancet* 1987; **1**: 289–291.
3. Danpure CJ, Jennings PR, Fryer P *et al.* Primary hyperoxaluria type I: genotypic and phenotypic heterogeneity. *J Inher Metab Dis* 1994; **17**: 487–499.
4. Hoppe B, Jahnen A, Bach D, Hesse A. Urinary calcium-oxalate saturation in healthy infants and children. *J Urol* 1997; **158**: 557–559.
5. Leumann E, Hoppe B, Neuhaus T, Blau N. Efficacy of oral citrate administration in primary hyperoxaluria. *Nephrol Dial Transplant* 1995; **10**(Suppl 8): 14–16.
6. Milliner DS, Eickholt JT, Bergstralh EJ *et al.* Results of long term treatment with orthophosphate and pyridoxine in patients with primary hyperoxaluria. *N Engl J Med* 1994; **331**: 1553–1558.
7. Hoppe B, Latta K, von Schnakenburg C, Kemper MJ, on behalf of the Arbeitsgemeinschaft für pädiatrische Nephrologie. Primary hyperoxaluria: the German experience. *Am J Nephrol* 2005; **25**: 276–281.
8. Allison MJ, Dawson KA, Mayberry WR, Foss JG. *Oxalobacter formigenes* gen. nov. sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. *Arch Microbiol* 1985; **141**: 1–7.
9. Abe K, Ruan ZS, Maloney PC. Cloning, sequencing and expression of OxlT, the oxalate:formiate exchange protein of *Oxalobacter formigenes*. *J Biol Chem* 1996; **271**: 6789–6793.
10. Lung HY, Baetz AL, Peck AB. Molecular cloning DNA sequence and gene expression of the oxalyl-CoA decarboxylase gene, *oxc*, from the bacterium *Oxalobacter formigenes*. *J. Bacteriol* 1994; **176**: 2468–2472.
11. Sidhu H, Ogden SD, Lung HY *et al.* DNA sequencing and expression of formyl-CoA transferase gene, *frc*, from *Oxalobacter formigenes*. *J Bacteriol* 1997; **179**: 3378–3381.
12. Sidhu H, Yenatska L, Ogden SD *et al.* Natural colonization of children in the Ukraine with the intestinal bacterium, *Oxalobacter formigenes*, using a PCR-based detection system. *Mol Diagn* 1997; **2**: 89–97.
13. Sidhu H, Schmidt ME, Cornelius JG *et al.* Direct correlation between hyperoxaluria/oxalate stone disease and the absence of the gastrointestinal tract dwelling bacterium *Oxalobacter formigenes*: possible prevention by gut recolonization or enzyme replacement therapy. *J Am Soc Nephrol* 1999; **10**(Suppl 14): S334.
14. Kumar R, Mukherjee M, Bhandari M *et al.* Role of *Oxalobacter formigenes* in calcium oxalate stone disease: a study from North India. *Eur Urol* 2002; **41**: 318–322.
15. Kwak C, Jeong BC, Lee JH *et al.* Molecular identification of *Oxalobacter formigenes* with the polymerase chain reaction in fresh or frozen fecal samples. *BJU* 2001; **88**: 627–632.
16. Kwak C, Jeong BC, Kim HK *et al.* Molecular epidemiology of fecal *Oxalobacter formigenes* in healthy adults living in Seoul, Korea. *J Endourol* 2003; **88**: 627–632.
17. Sidhu H, Hoppe B, Hesse A *et al.* Absence of *Oxalobacter formigenes* in cystic fibrosis patients: a risk factor for hyperoxaluria. *Lancet* 1998; **352**: 1026–1028.
18. Kleinschmidt K, Mahlmann A, Hautmann R. Anaerobic oxalate-degrading bacteria in the gut decrease fecal and urinary oxalate concentrations in stone formers. In: Ryall R, Bais R, Marshall VR, eds. *Urolithiasis 2*. Plenum Press: New York, USA, 1993: 439–441.
19. Kumar R, Ghoshal UC, Mittal RD. Infrequency of colonization with *Oxalobacter formigenes* in inflammatory bowel disease: possible role in renal stone formation. *J Gastro Hepatol* 2004; **19**: 1403–1409.
20. Troxel SA, Sidhu H, Kaul P, Low RK. Intestinal *Oxalobacter formigenes* colonization and urinary oxalate levels in calcium oxalate stone formers. *J Endourol* 2003; **17**: 173–176.
21. Sidhu H, Allison MJ, Chow JM *et al.* Rapid reversal of hyperoxaluria in a rat model following probiotic administration of *Oxalobacter formigenes*. *J Urol* 2001; **166**: 1487–1491.
22. Hatch M, Freel RW, Vaziri ND. Regulatory aspects of oxalate secretion in enteric oxalate elimination. *J Am Soc Nephrol* 1999; **10**(Suppl 14): S324–S328.
23. Hatch M, Cornelius J, Allison M *et al.* Oxalobacter sp. reduces urinary oxalate excretion promoting enteric oxalate excretion. *Kidney Int* 2006; **69**: 1–8.
24. Von Unruh GE, Langer MAW, Paar DW, Hesse A. Mass spectrometric-selected ion monitoring assay for an oxalate absorption test applying [$^{13}C_2$]oxalate. *J Chromatogr B* 1998; **716**: 343–349.
25. Hoppe B, Graf D, Offner G *et al.* Oxalic acid elimination in children with chronic renal failure: comparison between hemodialysis and peritoneal dialysis. *Pediatr Nephrol* 1996; **10**: 488–492.
26. Marangella M, Petrarulo M, Cosseddu D *et al.* Oxalate balance studies in patients on hemodialysis for type I primary hyperoxaluria. *Am J Kidney Dis* 1992; **19**: 546–553.
27. Bunchman TE, Swartz RD. Oxalate removal in type I hyperoxaluria or acquired oxalosis using HD and equilibration PD. *Perit Dial Int* 1994; **14**: 81–84.
28. Saxelin M, Tanja P, Salminen S. Fecal recovery following oral administration of *Lactobacilli casie* strain GG in gelatine capsules to healthy volunteers. *Int J Food Microbiol* 1995; **25**: 199–203.
29. Songisepp E, Kals J, Kullisar T *et al.* Evaluation of functional efficacy of an antioxidative probiotic in healthy volunteers. *Nutr J* 2005; **4**: 22–31.
30. Tannock GW, Munro K, Harmsen HJM *et al.* Analysis of fecal microflora of human subjects consuming probiotic containing L. rhamnosus DR 20. *Appl Environ Microbiol* 2000; **66**: 2578–2588.
31. Hesse A, Classen A, Knoll M *et al.* Dependence of urine composition on the age and sex of healthy subjects. *Clin Chim Acta* 1986; **160**: 79–86.
32. Hoppe B, Kemper MJ, Hvizd MG *et al.* Simultaneous determination of oxalate, citrate and sulfate in children's plasma with ion-chromatography. *Kidney Int* 1998; **53**: 1348–1352.