Urol Res (2009) 37:101–105
DOI 10.1007/s00240-009-0179-3

ORIGINAL PAPER

Plasma oxalate level in pediatric calcium stone formers with or without secondary hyperoxaluria

Przemysław Sikora · Bodo Beck · Małgorzata Zajączkowska · Bernd Hoppe

Received: 24 August 2008 / Accepted: 12 January 2009 / Published online: 30 January 2009

Abstract Plasma oxalate (POx) concentration is significantly elevated in primary hyperoxaluria, severe renal failure or ethylene glycol poisoning. In these conditions, the degree of hyperoxalemia correlates with the severity of systemic calcium oxalate (CaOx) deposition and should be therefore carefully monitored. Although secondary hyperoxaluria (secHyOx) is a common finding in pediatric patients with kidney stone disease, very little is known about POx in this condition. We therefore evaluated POx level in 59 children and adolescence with calcium urolithiasis (34 confirmed by CaOx stone analysis and 25 children with a strong clinical suspicion of this type of urolithiasis), with or without "mild" secHyOx. A control group consisted of 41 healthy sex- and age-matched children. We found that POx was significantly increased in children with calcium urolithiasis and secHyOx compared to healthy children (9.16 ± 3.60 vs. 6.42 ± 2.53 μmol/l), but that was not the case in children with calcium urolithiasis but with normal urinary oxalate excretion (7.12 ± 3.33 μmol/l). We conclude that POx may be slightly increased in some pediatric calcium stone formers with secHyOx, probably related to intestinal oxalate hyperabsorption.

Keywords Plasma oxalate · Urolithiasis · Hyperoxaluria · Children

P. Sikora (✉) · M. Zajączkowska
Department of Pediatric Nephrology,
Lublin Medical University, Chodźki 2, 20-093 Lublin, Poland
e-mail: sikoraprzem@hotmail.com

B. Beck · B. Hoppe
Division of Pediatric Nephrology,
Department of Pediatrics,
University Hospital Cologne, Cologne, Germany

Introduction

Oxalic acid is a simple dicarboxylic acid which is widely found in plants and in a lesser degree in animal tissues. In humans, however, it is an end product of glycine and glyoxylate metabolism and appears to have only minor physiological importance.

The clinical significance of oxalic acid results from the property of its anion (oxalate) to form insoluble salts with calcium cations at physiological pH levels of biological fluids [1]. This is particularly visible in conditions of an elevated urinary oxalate excretion, i.e. in the primary (types 1 and 2) and the secondary hyperoxalurias (secHyOx). The former are very rare, inherited systemic metabolic disorders characterized by enormous endogenous oxalate overproduction and thus huge hyperoxaluria (usually >1.0 mmol/24 h per 1.73 m^2), whereas in the latter condition, the urinary oxalate excretion is only slightly elevated (0.5–1.0 mmol/24 h per 1.73 m^2) as a result of its increased intestinal absorption or excessive dietary intake. Both forms of hyperoxaluria lead to calcium oxalate (CaOx) urolithiasis or nephrocalcinosis but the consequences of the primary hyperoxalurias are more dramatic, including multiorgan injury and renal failure [2]. Therefore, an assessment of urinary oxalate excretion is strongly recommended as part of the metabolic evaluation of patients with urolithiasis and/or nephrocalcinosis [2, 3].

Although the determination of urinary oxalate concentration is relatively simple and widely used, a measurement of plasma oxalate (POx) level is less available [1]. Hence, it is mostly determined as part of studies on oxalate homeostasis in the primary hyperoxalurias or chronic renal failure [4–7]. On the contrary, the determination of POx concentration in patients with urolithiasis was made only exceptionally, and the results were conflicting [8–12].

Therefore, we evaluated POx levels in a large group of children with this urolithiasis with or without secHyOx.

Materials and methods

The study comprised 59 children and adolescence (33 boys and 26 girls) aged 4.3–18 years, mean: 13.7 ± 3.6 years, with calcium urolithiasis. In 34 (57.6%) of them, infrared spectroscopy revealed CaOx stones (Whewellite, Weddellite, pure or mixed). In the remaining children, an assessment of stone composition was unfortunately impossible, e.g. due to stone loss after lithotripsy or sudden stone passage at home. However, in those children, CaOx urolithiasis was still suspected because all calculi were radiopaque and other possible types of stones (infection-related or cystine stones) were clinically and metabolically excluded. This was also observed in our series of 60 radioopaque stones obtained from our patients in the last 3 years, which had revealed CaOx in 89% and apatite in only 5.1% of stones (unpublished data). Recently, such as a small proportion of calcium phosphate stones in children with kidney stone disease was also reported by other authors [13]. However, due to remaining uncertainty as to chemical contents of stones in a part of our patients, we decided to classify all patients from the study group as calcium stone formers.

In the study group, 18 patients (15 boys and 3 girls) aged 7.7–18 years, mean: 13.7 ± 2.9 years were diagnosed to have "mild" secHyOx (urinary oxalate excretion >0.5 mmol/24 h per 1.73 m^2, range 0.509–0.975 mmol/24 h per 1.73 m^2), whereas the remaining 41 children (18 boys and 23 girls) aged 4.3–18 years, mean: 13.7 ± 3.9 years showed normal urinary oxalate excretion (range 0.100–0.494 mmol/24 h per 1.73 m^2). Urinary oxalate excretion in one patient was considerably high (0.975 mmol/24 h per 1.73 m^2), hence, in the range of patients with primary hyperoxaluria. However, as the clinical course was mild, the further measurements of urinary oxalate excretion in this patient were lower (0.600–0.750 mmol/24 h per 1.73 m^2) and the intestinal [$^{13}C_2$]oxalate absorption was high with 32.6%, we diagnosed secHyOx of unknown origin because no history or present symptoms of a gastrointestinal disorder associated with enteric hyperoxaluria (inflammatory bowel disease, short bowel syndrome) was found.

All patients had normal renal function expressed as creatinine clearance, calculated according to the Schwartz formula [14]. They were also not treated with antibiotics in the previous 3 months. During the study, their diet was unrestricted but like all our patients with urolithiasis, they asked to avoid foodstuffs rich in oxalate (e.g. spinach, rhubarb, beet root or ice tea). Medication influencing the oxalate homeostasis (e.g. Vitamin B6) was not administered.

A group of 41 healthy children and adolescence (22 boys and 19 girls) aged 4.2–18 years, mean 13.8 ± 3.4 years, served as a control group for the evaluation of POx concentration. They were selected from patients who were admitted to our hospital for scheduled minor surgery. All of them had no a history of urolithiasis. They had normal kidney function, did not receive any medication during the study and were not treated with antibiotics in the previous 3 months.

In the study group as well as in controls, blood samples for oxalate measurement were obtained in the morning under fasting conditions. The blood samples in the control group were obtained simultaneously with other routine laboratory tests prior to surgery.

To avoid in vitro neogenesis of oxalate, lithium-heparinized blood (3 ml) was placed directly on ice and was immediately centrifuged at 1,000×*g* for 5 min at 4°C. Obtained plasma was placed in the outer chamber of a Centrisart I ultrafiltration vial (Sartorius AG, Germany) and 40 µl of 1 M HCl per ml plasma was added in the inner chamber to ensure simultaneous acidification of ultrafiltrate during centrifugation (pH < 1.8). Plasma was then ultrafiltered at 1,500×*g* for 20 min at 4°C. Subsequently, all plasma samples were frozen and stored at −20°C until analysis but no longer than 1 month. POx concentration was measured with an ion chromatography system (DX-500; Dionex Corp., Sunnyvale, CA, USA) equipped with an analytical column (AS11, 2 mm) and a guard-column (AG11, 2 mm) as the stationary phase. The mobile phase was a KOH solution produced by an eluent generator (EG40, Dionex, USA). For that purpose, H_2O was continuously degassed with helium and KOH was run as an increasing gradient through the analysis. The eluent background conductivity was suppressed with an anion self-regenerating suppressor (ASRS-300, Dionex Corp., USA) to a level below 3 µS at the highest KOH concentration. Prior to analysis, plasma samples were diluted with 0.3 mM boric acid (1:5 or 1:10). To calculate POx concentration, a computer-based software (Chromeleon 6.3, Dionex Corp.) was used.

Urinary oxalate was measured enzymatically using oxalate oxidase (Oxalate KIT, Trinity Biotech, Ireland).

The statistical analysis was performed by the software STATISTICA (StatSoft Inc., Tulusa OK, USA) for Windows, version 7.1. Because values of POx levels were normally distributed according to the Shapiro–Wilk *W* test, they were expressed as means ± SD and group comparison was carried out with the Student's *t* test. Correlations were tested with the Spearman test. *P* values <0.05 were considered statistically significant.

The study protocol was approved by the ethics committee of the Medical University of Lublin and written consent was obtained from parents and patients.

Table 1 Mean (±SD) plasma oxalate concentration (μmol/l) in children with calcium urolithiasis and in healthy controls

Patients with calcium urolithiasis			Controls		
Boys ($n = 33$)	Girls ($n = 26$)	Total ($n = 59$)	Boys ($n = 19$)	Girls ($n = 22$)	Total ($n = 41$)
7.82 ± 3.10	7.64 ± 4.04	7.74 ± 3.52[a]	6.57 ± 2.73	6.27 ± 2.40	6.42 ± 2.53

[a] $P < 0.05$ patients with calcium urolithiasis (total) vs. controls (total)

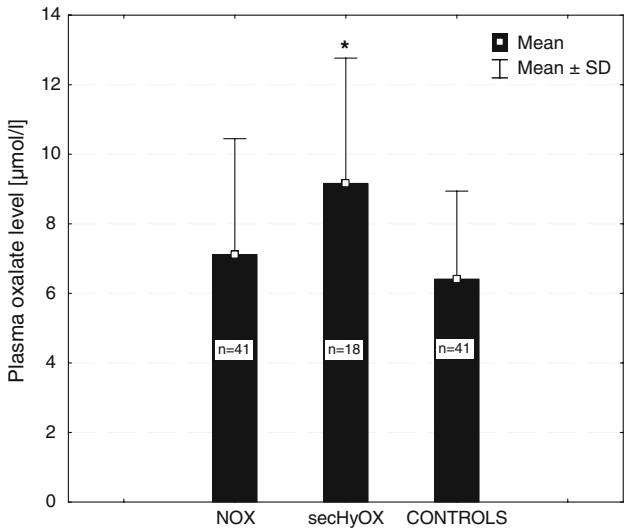

Fig. 1 Plasma oxalate concentration in children with calcium urolithiasis and normooxaluria (NOX) or secondary hyperoxaluria (secHyOx) and in healthy controls (*secHyOx vs. controls, $P < 0.01$; secHyOx vs. NOX, $P < 0.05$)

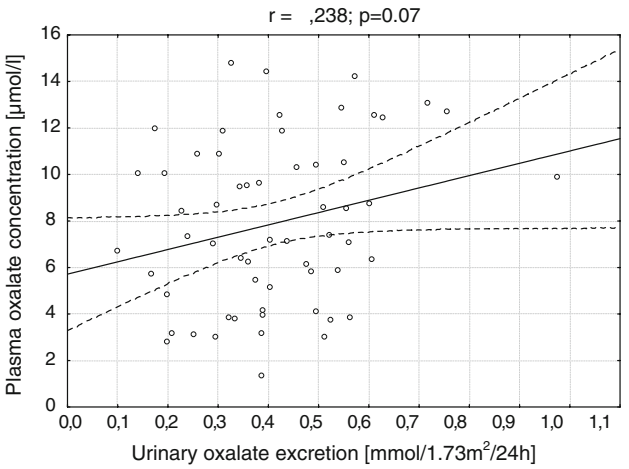

Fig. 2 Correlation between plasma oxalate concentration and urinary oxalate excretion in pediatric patients with calcium urolithiasis

Results

The mean POx concentration was significantly higher in patients with urolithiasis than in healthy children (7.74 ± 3.52 vs. 6.42 ± 2.53 μmol/l, $P < 0.05$, Table 1). In healthy children as well as in patients, the POx concentrations did not show a sex-dependent difference. However, in both groups, the mean POx level was slightly higher in boys. The highest POx levels were detected in the patients with urolithiasis and secHyOx. They were significantly higher as compared to both healthy children and children with urolithiasis but normooxaluria (NOX): 9.16 ± 3.60 versus 6.42 ± 2.53 μmol/l, $P < 0.01$ and 9.16 ± 3.60 versus 7.12 ± 3.33 μmol/l, $P < 0.05$, respectively (Fig. 1). The mean POx concentration in children with urolithiasis but NOX did not differ significantly from that in healthy controls. The correlation between POx concentration and urinary oxalate excretion was not statistically significant but both parameters tended to be positively related—correlation coefficient, $r = 0.238$; $P = 0.07$ (Fig. 2). There was no statistically significant correlation between the creatinine clearance and POx concentration in patients with urolithiasis ($r = 0.238$; $P = 0.07$).

Discussion

Calcium-containing calculi constitutes the absolute majority of urinary stones in adults and children in the western hemisphere. Up to 80% of them are composed of CaOx mono- or dihydrate or are found to be mixed forms [15, 16].

The pathogenesis of CaOx urolithiasis is complex and still a matter of investigation. Although hyperoxaluria is said to be less common than hypercalciuria as a single risk factor, urinary oxalate is obviously the more potent lithogenic factor [17, 18].

Different aspects of the oxalate metabolism in CaOx stone formers provoke a lot of interest. Although our knowledge of urinary oxalate excretion and particularly on intestinal oxalate absorption has recently increased, very little data on POx concentrations neither in the different forms of hyperoxaluria nor in CaOx urolithiasis per se are available.

The reason for this could be the methodological problems with POx measurement, particularly due to its very low concentrations (in the micromolar range) and its biochemical sensitivity. Therefore, the determination of POx concentration was carried out almost exclusively in patients with primary hyperoxaluria and renal insufficiency, regardless of its etiology [1, 4–7]. In both conditions, POx

concentration is considerably elevated and associated with clinical consequences (systemic oxalate deposition) that makes its monitoring very useful.

Reports on POx concentration in patients with idiopathic CaOx urolithiasis are sparse and the results are incongruent [8–12]. Some authors observed higher POx levels in stone formers in comparison to healthy controls [8, 12] while others did not [9–11]. In our study, we found a significantly increased POx concentration in children with CaOx urolithiasis and secHyOx in comparison to healthy children, as well as to children with CaOx urolithiasis but normal urinary oxalate excretion. Although the POx levels in the former group are lower as compared to those in patients with primary hyperoxaluria [6, 7], the significant differences may nevertheless provide specific new evidence for the pathophysiological basis of stone disease in this group of patients.

Before comparing our data concretely with previous findings, we recorded various methodological differences. First, numerous methods for POx determinations including gas chromatography [9], enzymatic assays [8, 12, 19], capillary electrophoresis [11] and ion chromatography were used [10], leading to numerous "normal" values. All abovementioned methods may theoretically give different and not entirely comparable results. Even ion chromatography, which seems to be one of the most reliable techniques for POx determination, is strongly influenced by sample handling and plasma preparation before analysis [20]. Therefore, the physiological range in adults is wide and was reported from as low as 0.7 to 2.9 μmol/l [10], and as high as 6.75 ± 2.62 μmol/l [4]. The values obtained in our control group are located near the upper limit of this wide range of supposedly normal values and they are comparable with previously published POx concentrations determined by the same method in healthy children (6.3 ± 1.09 μmol/l) [21].

Secondly and in contrast to our study, none of the previous investigations searched for differences in POx levels according to urinary oxalate excretion values. We can only speculate on possible mechanisms of an increased POx concentration in our patients with urolithiasis and secHyOx. Theoretically, the POx level is influenced by a variety of factors including endogenous production, intestinal absorption and renal oxalate clearance. Since in stone patients the latter resembles that in healthy subjects [9–11] and patients without primary hyperoxaluria supposedly have a normal endogenous oxalate production, the intestinal oxalate absorption could be a significant variable. Recently, we found using [$^{13}C_2$]oxalate absorption test that 38.3% of pediatric CaOx stone formers showed an intestinal oxalate hyperabsorption. Furthermore, in this group of patients, we confirmed a positive correlation between intestinal oxalate absorption and urinary oxalate excretion [22]. Unfortunately, for organizational reasons, we could not measure POx concentration at the time of [$^{13}C_2$]oxalate absorption test to assess a relation between both these parameters. Although in the current study, the relation between POx concentration and urinary oxalate excretion was not statistically significant, both parameters tended to be positively correlated (correlation coefficient 0.238; $P = 0.07$). Therefore, we suppose that a larger number of samples could confirm this relationship.

However, there were other observations already reported, which might support our hypothesis. For example, an influence of dietary oxalate ingestion on POx levels was reported. A significant increase in POx levels was found in CaOx stone formers as compared to healthy controls after a spinach meal [9]. An elevated POx level was also found in patients with cystic fibrosis who developed absorptive hyperoxaluria due to fat malabsorption and the absence of intestinal oxalate-degrading bacteria such as *Oxalobacter formigenes* [23]. Unfortunately, POx levels in other enteric conditions leading to hyperoxaluria, e.g. jejunoileal bypass, small bowel resection and chronic inflammatory bowel diseases are not available as yet. It would be particularly interesting and potentially clinically useful to obtain such data, because these diseases may lead to oxalate nephropathy, renal failure and even to severe systemic oxalosis [24–26].

Conclusions

Plasma oxalate concentration may be slightly increased in some pediatric calcium stone formers with secHyOx, probably resulting from intestinal oxalate hyperabsorption. However, this finding seems to be of limited clinical value in individual patients, due to modest differences in POx level and considerable laboratory requirements for POx measurement. Thus, the determination of POx concentration in this population may have more theoretical values, providing insights into the underlying mechanisms of hyperoxaluria.

Acknowledgments The research was supported by Polish State Committee for Scientific Research KBN grant 2P05D 117 26.
The authors thank Mrs. U. Biaduń and Mrs. M. Wawrzyszuk for their great technical support.

References

1. Chalmers RA, Purkiss P (1991) Oxalic acid in plasma and urine. In: Hommes FA (ed) Techniques in diagnostic human biochemical genetics: a laboratory manual. Wiley, New York, pp 359–376
2. Leumann E, Hoppe B (2001) The primary hyperoxalurias. J Am Soc Nephrol 12:1986–1993
3. Milliner DS (2005) The primary hyperoxalurias: an algorithm for diagnosis. Am J Nephrol 25:154–160. doi:10.1159/000085407

4. Petrarulo M, Bianco O, Marangella M, Pellegrino S, Linari F, Mentasti E (1990) Ion chromatographic determination of plasma oxalate in healthy subjects, in patients with chronic renal failure and in cases of hyperoxaluric syndromes. J Chromatogr A 511:223–231. doi:10.1016/S0021-9673(01)93286-8
5. Kasidas GP, Nemat S, Rose GA (1990) Plasma oxalate and creatinine and oxalate/creatinine clearance ratios in normal subjects and in primary hyperoxaluria. Evidence for renal hyperoxaluria. Clin Chim Acta 191:67–78. doi:10.1016/0009-8981(90)90059-2
6. Hoppe B, Kemper MJ, Bökenkamp A, Langman CB (1998) Plasma calcium-oxalate saturation in children with renal insufficiency and in children with primary hyperoxaluria. Kidney Int 54:921–925. doi:10.1046/j.1523-1755.1998.00066.x
7. Hoppe B, Kemper MJ, Bökenkamp A, Portale AA, Cohn RA, Langman CB (1999) Plasma calcium oxalate supersaturation in children with primary hyperoxaluria and end-stage renal failure. Kidney Int 56:268–274. doi:10.1046/j.1523-1755.1999.00546.x
8. Butz M, Kohlbecker G (1980) Oxalate urolithiasis: significance of serum and urinary oxalate. Urol Int 35:303–308
9. Hoshina A (1984) Plasma oxalate concentration in calcium oxalate stone formers. Hinyokika Kiyo 30:1405–1415
10. Schwille PO, Manoharan R, Rümenapf G, Wölfel G, Berens H (1989) Oxalate measurement in the picomol range by ion chromatography: values in fasting plasma and urine of controls and patients with idiopathic urolithiasis. J Clin Chem Clin Biochem 27:87–96
11. Ogura H (2000) Determinations of oxalate in urine and plasma by capillary electrophoresis. Nippon Hinyokika Gakkai Zasshi 91:547–555
12. Jadeszko I, Porowski T, Zoch-Zwierz WM, Wasilewska AM, Hackiewicz L (2005) Assessment of oxalate concentration in serum and urine of children with renal stones. Wiad Lek 58(Suppl 1):20–24
13. DeFoor W, Minevich E, Jackson E, Reddy P, Clark C, Sheldon C, Asplin J (2008) Urinary metabolic evaluations in solitary and recurrent stone forming children. J Urol 179:2369–2372. doi:10.1016/ j.juro.2008.01.151
14. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A (1976) A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 58:259–263
15. Milliner DS, Murphy ME (1993) Urolithiasis in pediatric patients. Mayo Clin Proc 68:241–248
16. Lewandowski S, Rodgers AL (2004) Idiopathic calcium oxalate urolithiasis: risk factors and conservative treatment. Clin Chim Acta 345:17–34. doi:10.1016/j.cccn.2004.03.009
17. Robertson WG, Peacock M (1980) The cause of idiopathic calcium stone disease: hypercalciuria or hyperoxaluria. Nephron 26:105–110
18. Smith LH (1991) Diet and hyperoxaluria in the syndrome of idiopathic calcium oxalate urolithiasis. Am J Kidney Dis 17:370–375
19. Porowski T, Zoch-Zwierz W, Konstantynowicz J, Korzeniecka-Kozerska A, Michaluk-Skutnik J, Porowska H (2008) Reference values of plasma oxalate in children and adolescents. Pediatr Nephrol (in press)
20. Petrarulo M, Cerelli E, Marangella M, Maglienti F, Linari F (1993) Ion-chromatographic determination of plasma oxalate reexamined. Clin Chem 39:537–539
21. Hoppe B, Kemper MJ, Hvizd MG, Sailer DE, Langman CB (1998) Simultaneous determination of oxalate, citrate and sulfate in children's plasma with ion chromatography. Kidney Int 53:1348–1352. doi:10.1046/j.1523-1755.1998.00891.x
22. Sikora P, von Unruh GE, Beck B, Feldkötter M, Zajączkowska M, Hoppe B (2008) [$^{13}C_2$]oxalate absorption in children with idiopathic calcium oxalate urolithiasis or primary hyperoxaluria. Kidney Int 73:1181–1186. doi:10.1038/ki.2008.63
23. Hoppe B, von Unruh GE, Blank G, Rietschel E, Sidhu H, Laube N, Hesse A (2005) Absorptive hyperoxaluria leads to an increased risk for urolithiasis or nephrocalcinosis in cystic fibrosis. Am J Kidney Dis 46:440–445. doi:10.1053/j.ajkd.2005.06.003
24. Wharton R, D'Agati V, Magun AM, Whitlock R, Kunis CL, Appel GB (1990) Acute deterioration of renal function associated with enteric hyperoxaluria. Clin Nephrol 34:116–121
25. Kiss D, Meier R, Gyr K, Wegmann W (1992) Sekundäre Oxalose nach Dündarmresektion mit Niereninsuffizienz und Oxalatvaskulopathie. Schweiz Med Wschr 122:854–857
26. Hassan I, Juncos LA, Milliner DS, Sarmiento JM, Sarr MG (2001) Chronic renal failure secondary to oxalate nephropathy: a preventable complication after jejunoileal bypass. Mayo Clin Proc 76:758–760