and Calcium Oxalate Stone Disease

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Oxalosis

and Calcium Oxalate Stone Disease

Speaker Abstracts

Animal Model of Hyperoxaluria: Experimental Induction

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Introduction: Clinically, hyperoxaluria is caused by genetic disorders, such as primary hyperoxaluria, which result in disturbance of oxalate biosynthesis, increased availability of oxalate precursors such as ethylene glycol poisoning and methyoxyflurane anesthesia, and intestinal hyperabsorption of oxalate as occurs in enteric hyperoxaluria and mild metabolic hyperoxaluria. These disorders, individually or in combination with other factors, can induce calcium oxalate (CaOx) crystallization in the kidneys and eventually nephrolithiasis.

Experimental Induction of Hyperoxaluria: The role of hyperoxaluria in CaOx nephrolithiasis is generally investigated by employing physicochemical, tissue culture and animal models. Most animal model studies require experimental induction of hyperoxaluria. In our studies 0.75% aqueous solution of ethylene glycol is administered as drinking water to male Sprage-Dawley rats.[1] Rats become hyperoxaluric by day 3. CaOx crystalluria follows within the next 2 to 3 weeks and 9 of 10 rats become nephrolithic within 4 to 6 weeks. Large crystal deposits or nephroliths of CaOx mono and dihydrate crystals are seen at the renal papillary tips and calyces. Urinary pH goes down and volume goes up. Rats also become hypocitraturic and hypomagnesuric.

At this time the kidneys remain functionally normal as indicated by urinary creatinine levels, but urinary excretion of many enzymes such as lactate dehydrogenase, and N-acetyl-ß-glucoseaminidase is significantly increased.[2,3] In addition lipid peroxides are significantly elevated in both the kidneys and urine indicating free radical induced injury.

The lipid profile of cell membranes is also altered. Brush border membranes contain high amounts of cholesterol, a hallmark of cellular adaptive response and the development of cytoresistance.[4] Sphingomyelin levels are also increased suggestive of the participation of sphingomyelin signal transduction pathway.[5]

Morphological examination of the kidneys shows damage to some renal epithelial cells.[6] Cells of proximal tubules and those containing crystals appear more sensitive, exhibiting intracellular edema, widened intracellular spaces, dividing nuclei and clubbing of microvilli. Crystals are mostly intraluminal and generally associated with membranous vesicles. A few appear directly attached to the epithelial cell surfaces. Some crystals are present inside the cells while a few are also present in the interstitium. Inflammatory cells such as monocytes, macrophages, and polymorphonuclear leukocytes surround interstitial crystals. Deposits at the renal papillary tips include sub-epithelial crystals anchored to the basement membrane.

Hyperoxaluria and crystal deposition are associated with increased production of certain macromolecules such as bikunin (BK)[7] and osteopontin (OPN).[8,9] However there is no increase in the production of

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Tamm-Horsfall (THP) protein. Both BK and OPN are involved in inflammatory responses and their increased production by renal epithelial cells again points to cells responding to the presence of high levels of oxalate and/or CaOx crystals.

Nephroliths are composed of crystals and organic matrix. The matrix is present both within the crystals and between them and contains lipids, proteins and carbohydrates. Ultrastructural immunolocalization shows the intercrystalline matrix to be mostly OPN while intracrystalline matrix contains lipids, THP, albumin and perhaps many others that, to date, have not been investigated.

Concluding Remarks: The experimental model of hyperoxaluria described here has provided new insights into the role of oxalate in CaOx crystal deposition in the kidneys. Evidence is provided that oxalate is not an inert useless substance, with importance only in increasing the urinary CaOx supersaturation. As can be seen, oxalate, with or without CaOx crystals, is able to injure the cells and provoke an adaptive response. The balance between injurious and adaptive factors may decide the course of events in which concentration of oxalate and duration of exposure plays significant role.

We have utilized the experimental model to investigate the interaction between renal epithelial cells and oxalate and/or CaOx crystals in order to understand the pathogenesis of CaOx nephrolithiasis. A modification of the model is being used by Dr. Hatch to examine aspects of oxalate secretion in enteric oxalate elimination. Drs. Peck and Sidhu are utilizing a similar model to understand the role of *Oxalobacter formigenes* in reducing hyperoxaluria. We have also employed the model to study encrustation of catheter-like materials in the bladder. Thus this model can be adapted to any investigations requiring hyperoxaluric conditions in whole body situations.

References:

1. Khan SR, Glenton PA, 1995, *J Urol* 153:811.
2. Khan SR, Hackett RL, 1993, *Cont Nephrol* 101:190.
3. Thamilselvan S, Hackett RL, Khan SR, 1997, *J Urol* 157:1059.
4. Zager RA et al, 1999, *Kid Intl* 56:1788.
5. Zager RA, 1999, *J Am Soc Nephrol* 11:894.
6. Khan SR, 1995, *Scann Microsc* 9:89.
7. Iida S et al, 1999, *J Am Soc Nephrol* 10:986.
8. McKee MD, Nanci A, Khan SR, 1995, *J Bone Min Res* 10:1913.
9. Gokhale JA, McKee MD, Khan SR, 1996, *Urol Res* 24:201.

Are There Any Valid Animal Models of Primary Hyperoxaluria?

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The identification of relevant animals models, either naturally occurring or artificially produced in the laboratory, is an essential part of the process of developing rational treatments for human genetic diseases (1). In this respect the primary hyperoxalurias are no exception. However, extrapolation of the results obtained in these models to the situation in human patients can be fraught with difficulties because of genetic, metabolic or physiological differences between the species. Again, the primary hyperoxalurias are no exception. Many differences are known to exist between different species in the metabolic events that lead to oxalate synthesis, as well as in the way these species deal with the oxalate, once synthesised. For example, as far as metabolism is concerned, there are frequently differences in the overall levels of expression, developmental expression, tissue specificity, substrate specificity and subcellular distribution of at least some of the enzymes known to directly or indirectly determine the rate of oxalate synthesis. The best example of this inter-species variation is the enzyme alanine:glyoxylate aminotransferase (AGT) which, in humans at least, is recognised as being one of the major determinants of endogenous oxalate synthesis. The activity, substrate specificity and subcellular distribution of AGT all vary widely between different mammals (2). Unfortunately, some of the biggest differences occur between humans and laboratory animals, such as rats and mice.

Naturally occurring analogues of human diseases are not necessarily any more useful than laboratory created models. For example, ten years ago we identified primary hyperoxaluria type 1 in dogs (3) and primary hyperoxaluria type 2 in cats (4-5). However, their use as models was severely compromised by enzymic/metabolic differences (e.g., AGT subcellular distribution) as well as physiological differences (e.g., kidney structure and function).

Although it will be difficult, if not impossible, to identify perfect animal models for the human primary hyperoxalurias, it is clear that specific metabolic or pathophysiological processes involved in oxalate synthesis or pathophysiology can be duplicated in non-human mammals or even *in vitro* systems. Thus the use of animal models to investigate only specific aspects of the disease process (i.e. possibly using a different species or system for each process) might be the better way forward rather than to try to simulate human primary hyperoxaluria *in toto.*

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References

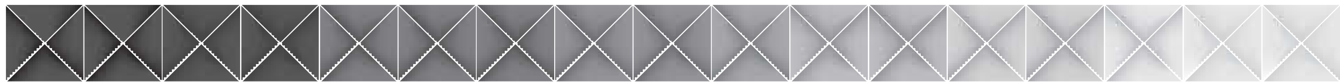
1. Danpure, C.J. (2000) Primary hyperoxaluria. In *"The Metabolic and Molecular Bases of Inherited Disease"*, 8th edition, McGraw-Hill, New York, eds. Scriver, C.R. et al., in press.

2. Danpure, C.J. (1997) Peroxisomal and mitochondrial targeting of alanine:glyoxylate aminotransferase in mammalian evolution and disease. *Bioessays,* 19, 317-326.

3. Danpure, C.J., Jennings, P.R. & Jansen, J.H. (1991) Enzymological characterization of a putative canine analogue of primary hyperoxaluria type 1. *Biochim. Biophys. Acta.* 1096, 134-138.

4. McKerrell, R.E., Blakemore, W.F., Heath, M.F., Plumb, J. Bennett, M.J. Pollitt, R.J. & Danpure, C.J. (1989) Primary hyperoxaluria (L-glyceric aciduria) in the cat: a newly recognised inherited disease. *Vet. Rec.* 125, 31-34.

5. Danpure, C.J., Jennings, P.R., Mistry, J., Chalmers, R.A., McKerrell, R.E., Blakemore, W.F. & Heath, M.F. (1989) Enzymological characterization of a feline analogue of primary hyperoxaluria type 2: a model for the human disease. *J. Inher. Metab. Dis.* 12, 403-414.



Oxalobacter formigenes: An Epidemiological Enigma

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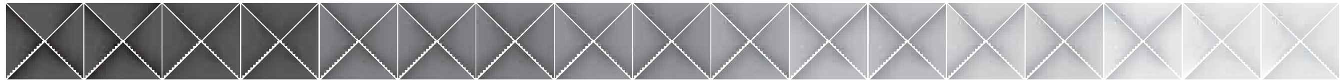
Oxalobacter formigenes, a gram-negative, anaerobic bacterium, inhabits the gastrointestinal tracts of most vertebrates, including man. This bacterium plays an important symbiotic relationship with its hosts by regulating oxalic acid homeostasis. Unlike other microorganisms possessing a capability to degrade oxalic acid, *Oxalobacter formigenes* is the only one known to rely totally on oxalic acid as an energy source. Analysis of isolates from various vertebrate species have identified multiple strains that are currently divided into two major subgroups; however, there appears to be no species-specificity. In initial epidemiological studies of *Oxalobacter formigenes* colonization in humans, several important observations emerged: humans become colonized as young children (usually between 1-3 years of age); virtually 100% of all children get colonized naturally; colonization is lost frequently in adult populations (world-wide colonization rates appear to be 60-80%); and, colonization is sensitive to diet and use of antibiotics.

Oxalobacter formigenes is a fastidious anaerobe whose exposure to oxygen, even for short periods of time, affects its viability. This raises an interesting question: "how is *Oxalobacter formigenes* transmitted between individuals?" To answer this question, female laboratory rats (known to be non-colonized) were colonized with either *Oxalobacter formigenes* group I strain OxWR or group II strain OxGP. Colonization was maintained with a high-oxalate diet. Each female was then mated to a non-colonized male. Fecal specimens collected from the offspring were tested for the presence of the appropriate strain of *Oxalobacter formigenes.* Similar to the human studies, *Oxalobacter formigenes* was undetectable in neonate rats up to 10 days post-partum. By day 14, 50% of the offspring, and by day 21 (normal time of weaning) 100% of the offspring were colonized with the bacterial strain carried by their mothers. To determine if *Oxalobacter formigenes* is transmitted vertically or horizontally, a similar experiment was performed except that the newborn rats were placed with foster mothers colonized with the opposite bacterial strain. While colonization occurred temporally in a similar manner, the offspring were colonized with the *Oxalobacter formigenes* strain of the foster mothers.

These data indicate that intestinal colonization is transmitted horizontally. How *Oxalobacter formigenes* survives the aerobic environment to be transmitted horizontally remains unknown.



The Dynamics of Intestinal Handling of Oxalate and the Role of Oxalobacter

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The long-term goal of our current investigations is to reduce the burden of oxalate excretion by the kidneys before the onset of oxalate-induced renal damage and/or failure. The aim is to enhance elimination of oxalate into the large intestine where it can be innocuously degraded by *Oxalobacter sp.,* a bacterium that resides exclusively in this segment of the alimentary tract, whose sole carbon source is oxalate. We propose that by maximizing the enteric excretion and luminal degradation of oxalate, renal excretion of oxalate will be decreased, hence the risks associated with hyperoxaluria can be minimized.

The notion that intestinal transport of oxalate has a role in oxalate homeostasis is now generally accepted on the basis of a number of our studies demonstrating the capacity of intestinal epithelia to regulate the magnitude and direction of oxalate movement. Furthermore, it is clear that the distal colonic segment is both the primary and terminal location for the enhanced oxalate absorption in *enteric hyperoxaluria* and the *adaptive* secretion and excretion of oxalate in chronic renal failure. The existence of epithelial transport systems for secreting oxalate into the lumen and the fact that these pathways can be locally regulated, suggest a direction for novel therapeutic approaches by exploiting luminal *Oxalobacter* or its products.

Recently, we hypothesized that *Oxalobacter* may possess a strategic ability to optimize substrate availability within the intestinal lumen. We postulated that the capacity to locally modulate epithelial oxalate transport could reasonably be expected because it would have survival value for this oxalate-dependent bacterium in the absence of dietary sources of oxalate. Specifically, the question was whether *Oxalobacter* elaborates a secretagogue that would locally induce mucosal oxalate secretion, in addition to its oxalate-degradative capabilities. In this regard, we tested the effects of select bacterial preparations on colonic oxalate transport *in vitro.* Intestinal oxalate handling was also examined in rats that were colonized with *Oxalobacter* compared with those that were not colonized. In addition, the effects of luminal *Oxalobacter* on urinary oxalate excretion was evaluated under conditions where dietary calcium was manipulated. The results of these experiments, as well as the results of another *in vivo* study examining the effects of administering an encapsulated *Oxalobacter* cell lysate/enzyme preparation to hyperoxaluric rats with chronic renal failure, support the concept that luminal *Oxalobacter* can indirectly regulate urinary oxalate excretion by promoting an enteric oxalate shunt.

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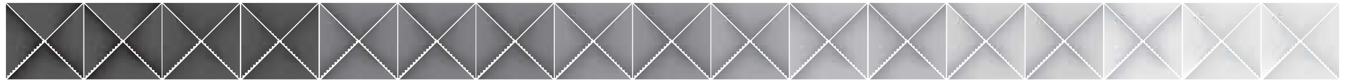

It should be noted, however, that the interpretation of some of our experimental results derived from rats artificially colonized with *Oxalobacter* is difficult because there were complications with sustaining colonization.

While these studies have revealed novel aspects associated with the dynamics between *Oxalobacter* and the transporting gut mucosa, they also underscore large gaps in our knowledge regarding the luminal factors involved in both initiating and maintaining colonization. Nonetheless, these results emphasize: a) the important physiological role of intestinal oxalate secretory pathways in shifting the balance of oxalate excretion between the renal and enteric routes of elimination and b), they provide direction for the production of a therapeutic supplement that might exploit the combined secretagogue and degradative actions of *Oxalobacter.*

Oxalobacter formigenes: A Probiotic?

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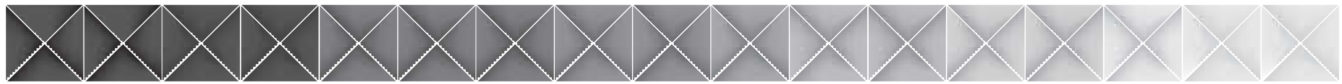
Oxalobacter formigenes [Oxf], an obligate anaerobe, was isolated in 1983 from the rumen of sheep. It was demonstrated early that the organism's sole energy source was oxalate. In turn it was shown that Oxf had the potential to protect sheep against hyperoxalosis, caused by excessive ingestion of oxalate in the diet. Oxf was then described in man, with colonic colony counts of 0 to $1x10^8$ c.f.u. with colonization of 100% of children and 70% of adults. We subsequently showed that Oxf was both present in volunteers and inducible in volunteers fed calcium oxalate.

We examined the hypothesis that if Oxf plays a physiologic role, then it was likely to be diminished or absent in conditions where there was hyperoxaluria. Enteric hyperoxaluria is such a condition, and we showed that in Crohn's disease, the prevalence of Oxf was 10%, ulcerative colitis was 20% and that it was not detected in 2 patients with steatorrhea. Controls had a prevalence of 71%. Furthermore oxalate degradation was very low in the patients compared with controls. The presence of a colon is required for the development of hyperoxaluria, implying that colonic absorption is required for this condition. Thus the absence of Oxf from the colon, may allow for higher colonic concentrations of oxalate, greater absorption and hence higher urinary levels.

Oxalate nephrolithiasis is a common painful condition. In at least 50 percent of patients it is recurrent. We have therefore extended the hypothesis to examine whether there is a reduced prevalence of Oxf in patients with recurrent stone disease. In spousal pairs the prevalence of Oxf was less in the patients [22%] than in the spouses [56%]. This data, along with other author's preliminary data, suggests that the hypothesis requires formal testing in a large scale epidemiological study. If the hypothesis is correct, then it would open the door to a new concept of treatment for hyperoxaluria associated nephrolithiasis, namely the colonization of the colon with Oxf or feeding of the relevant enzyme systems.



Commercial Applications of the Oxalate Degrading Bacterium, Oxalobacter formigenes

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O. formigenes is a gram-negative, anaerobic bacterium colonizing the gastrointestinal tracts of most vertebrate animals, including humans. Evidence suggests that this bacterium maintains an important symbiotic relationship with its hosts by regulating oxalic acid levels of the plasma and urine through the control of its intestinal absorption and enteric elimination. *O. formigenes,* either as whole live cells or as its enzymatic components, may offer a unique direction for controlling the body's burden of oxalate.

Ixion Biotechnology, Inc. is developing Ox-Control™ (a nutritional probiotic supplement) and IxC1-62/47 (an enzyme-based prescription drug) for the prevention of oxalate-related disorders. A recent study, conducted in the rat model of hyperoxaluria, showed the efficacy and safety of the probiotic supplement with live *O. formigenes.* Laboratory rats (n=30) were divided into six experimental groups: Group I rats were maintained on a regular diet; Group II rats received the regular diet supplemented with 1% ammonium oxalate to induce hyperoxaluria; Groups III, IV, V and VI rats were fed the regular diet supplemented with ammonium oxalate, but received a daily esophageal gavage of *O. formigenes* cell suspension at concentrations $1x10^3$ to $1x10^9$ cfu/ml. All rats receiving a probiotic bacterial supplement with their diets (i.e., Groups III to VI) exhibited decreased urinary oxalate excretions as compared to rats in Group II. The decreases in urinary oxalate concentrations were directly related to the dose of bacterium. By day 14, the urinary oxalate levels of rats in Groups V and VI had returned nearly to normal levels. The probiotic treatment of the rats for a period of 14 days and up to a dose of 10^9 cfu/day was well tolerated and resulted in no obvious pathological changes within any portion of the intestines. Thus, development of *O. formigenes* as a probiotic food supplement for the prevention of absorptive/enteric hyperoxaluria and its complications seems highly feasible.

The effectiveness of *O. formigenes* oxalate-degrading enzyme supplementation as enteric-coated capsules in reducing hyperoxaluria has also been tested in the rat model with results quite similar to those observed with the probiotic. The encapsulated enzymes in IxC-62/47 are being produced as both natural and as recombinant proteins. Large-scale production and purification of enzymes has already been achieved. Optimization of a formulation with dosing and toxicity evaluation studies is currently underway.

Intestinal Bacteria and Nephrolithiasis: Oxalobacter and Lactic Acid Bacteria

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The role of *Oxalobacter formigenes* colonization, its presence and absence, in prevention and causation of kidney stone formation remains very suggestive, but not proven. Koch's postulates, useful in proving that an organism causes a specific disease, are not easily adapted to the novel notion that the absence of elements of the normal flora may be causative of disease. Not all patients tested negative for *O. formigenes* have hyperoxaluria; clearly there are many possible variables. Genetic polymorphisms of the normal flora, as shown in studies of *Helicobacter pylori* and peptic ulcer disease, may be as important in disease causation as whether the organism is present or not.

One disease state used to investigate the role of *Oxalobacter* is cystic fibrosis, in which the absence of the organism seems to correlate with stone formation. Stone disease has a very low incidence in CF patients despite widespread antibiotic use and there are few data showing that the rate of stones or abnormalities in urine chemistries is different than in age-matched healthy controls. Several other hypotheses have been advanced to explain stone formation in CF patients, including hyperuricosuria attributed to contamination of pancreatic enzyme supplements or disordered metabolism; and hyperoxaluria due to catabolic abnormalities. In addition, mutations of chloride channels in Dents disease and Bartters syndrome are associated with stones or nephrocalcinosis, though these conditions appear to cause hypercalciuria which is not present in CF.

Other organisms may also play a role. A recent study demonstrated that some strains of lactic acid bacteria such as *Lactobacillus acidophilus* and *L. brevis, Streptococcus thermophiles* and *Bifidobacter infantis* can metabolize oxalate *in vitro,* though the organisms lack OxlT of *O. formigenes*. These organisms are found in foods, are categorized as GRAS, ("Generally regarded as safe") by FDA, and are well tolerated when administered orally. They have recently been shown to have important beneficial effects in inflammatory bowel disease. Six patients with a history of calcium oxalate stone formation and idiopathic hyperoxaluria were treated for one month with an oral preparation of these bacterial species. All six demonstrated a significant reduction in urinary oxalate excretion. We now plan a randomized controlled trial of this preparation's effect on urinary risk factors for stone disease.

Intracellular Oxalate Concentrations

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In recent years, the results from several laboratories have suggested that elevated levels of the free oxalate anion may have a role in the pathogenesis of renal disease. For example, relatively low concentrations of extracellular oxalate appear to be associated with alterations in cell cycle (apoptosis), initiation of free-radical production, and alteration of gene expression. One implication of these findings is the possibility that the free anion, rather than (or in addition to) calcium oxalate crystals, initiates these events by an intracellular mechanism. One way to evaluate these possibilities is to establish the level of cell oxalate. It would seem a relatively simple matter to provide a definitive answer to the question of what the intracellular concentration of oxalate is under normal and pathological conditions. Unfortunately, while we have a rather detailed knowledge of transmembrane oxalate transport mechanisms, including symport, antiport, and even channel pathways, how oxalate fluxes through these mechanisms are coordinated to provide a steady-state concentration of cytosolic oxalate has not been convincingly established. Examination of recent estimates of cellular oxalate levels in various cell types reveals a rather broad spectrum of concentrations; ranging from 25 to 500 µmol/l. While much of this variability is due to the differences in cell types, methods, and experimental conditions, this variance underscores the need to establish more precise thermodynamic criteria regarding transmembrane oxalate distributions. For the sake of argument, and as a quantitative means to interpret reported oxalate concentrations in cells, it is instructive to compute the equilibrium distribution of oxalate at nominal membrane potentials (-60 mV) and extracellular oxalate concentrations (5 µmol/l). Under such conditions and assuming a cell (like secretory enterocyte) exhibits a finite conductance for oxalate, then the minimum intracellular oxalate activity would be about 1µmol/l. It is noteworthy that low activity of cytosolic oxalate (~3 µmol/l) is sufficient to drive active oxalate secretion across intestinal epithelia under similar conditions. The discrepancies between these theoretical and measured intracellular oxalate concentrations might result from compartmentalization of the oxalate anion–a possibility that has been largely ignored but underscores the importance in making the distinction between intracellular oxalate contents and activities.

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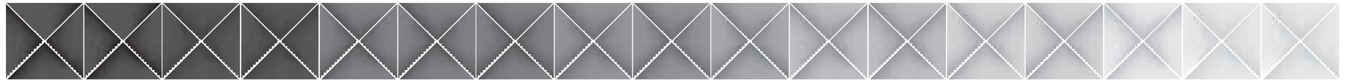

Intracellular Oxalate Concentrations

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We have recently piloted some fluorescence based techniques to evaluate intracellular oxalate activities. One approach has been to establish the effects of extracellular oxalate on receptor-mediated Ca^{2+} spikes, measured using Fura-2 fluorescence, in renal and intestinal epithelial cells. Remarkably, 400 µmol/l extracellular free oxalate produced significant depressions in Ca^{2+} spike height but had no effect on resting Ca^{2+} activity (in agreement with previous findings). Assuming this blunting of spike height results solely from oxalate buffering of released Ca^{2+}, we estimate cytosolic oxalate would be about 400 µmol/l—some 10 fold greater than that calculated from the equilibrium potential for oxalate (40 µmol/l) under these experimental conditions. Since these studies were performed with a calcium sensor with a low K_d, the magnitude of the Ca^{2+} spike is underestimated l—hence, the cytosolic oxalate activity is likely overestimated. Nevertheless, these findings suggest that when extracellular oxalate is elevated, cytosolic oxalate may indeed reach levels considerably greater than that predicted from equilibrium considerations thereby affecting Ca^{2+} signaling and perhaps other intracellular regulatory pathways.

Oxalate Measurement Methodologies

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For an adequate diagnosis of all kinds of hyperoxaluria, the primary and the secondary forms, it is of utmost importance to perform the necessary urine and plasma determinations with a good, simple and cost effective method, which also yields a high reliability and reproducibility. Before samples are analyzed adequate preservation and preparation are necessary. Bottles to collect urine samples should either be prepared with hydrochloric acid or thymol. Blood samples need a more rapid and specific preservation with HCL to exclude *in vitro* neogenesis of oxalate. Which urine and plasma determination is necessary? Next to urinary oxalate, both glycolate and L-glyceric acid should be analyzed to distinguish the two currently known types of primary hyperoxaluria. Furthermore, especially in patients with renal failure, plasma determinations of both oxalate and glycolate will be helpful to establish the diagnosis. Therefore, the optimal method would allow a simultaneous determination of these substances.

Predominantly, three methods are compared:

• Enzymatic kits (e.g. Sigma® Kit)

• Ion chromotography (IC)

• HPLC-enzyme reactor method

Concentrating on the following parameters:

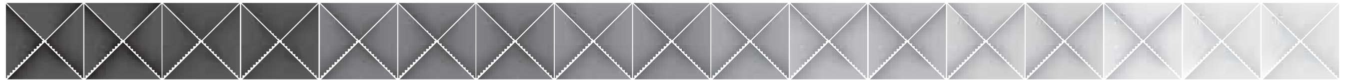
• simple and rapid procedure, low expenses for equipment, cost effectiveness

• reliability and reproducibility

• possibility of simultaneous determination of oxalate and glycolate.

The Sigma® Kit is easiest to apply, expensive equipment is not necessary. If the equipment is available, the IC- or the HPLC-method are cheaper per analysis. All three methods showed a good reliability and reproducibility with the lowest coefficient of variation (CV) for the Sigma® Kit in the urines with a low oxalate concentration (CV = 7.2 %). Ion chromatography, however, had the lowest CV (3.9 %), when urines with a high oxalate concentration were analyzed. The HPLC-enzyme reactor method achieved CV-levels from 2.2-6.7 %. Overall the recovery rate for all three methods was > 97 %. Simultaneous determination of both oxalate and glycolate, however, was only described for the IC-method.

In conclusion, all methods appear reproducible and reliable. Comparable results were achieved, when parallel determinations were performed. The Sigma® Kit seems to be the easiest to perform, no specific equipment is necessary and, leading the attention to the low CV for determination of low oxalate concentrations, it is surely an appropriate method for reliable determination of urinary oxalate. Ion-chromatography has, when the equipment is affordable, its main advantage in both the simultaneous determination not only of oxalate and glycolate, but as well of sulfate, phosphate and citrate. In addition, it becomes cheaper, the more samples are analyzed. Plasma oxalate (and glycolate) determination is also possible with the IC-method and the HPLC-enzyme reactor method.

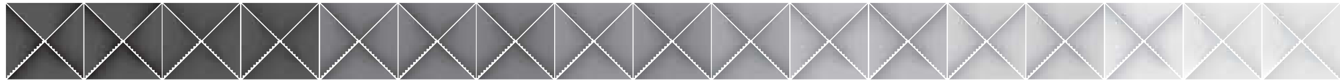


Oxalate as a Renal Toxin – The "Pro" Argument

C. Scheid

University of Massachusetts Medical School

A number of laboratories including our own have provided evidence indicating that high levels of oxalate are toxic for renal epithelial cells. This evidence includes the finding that exposure to oxalate concentrations ≥ 0.5 mM elicits an increase in membrane permeability to vital dyes (within 2-4 hr) and a decrease in cell numbers (within 1 day). This oxalate-induced increase in cell death appears to involve both apoptotic and necrotic cell death. Evidence for apoptotic cell death in renal cells includes the finding that oxalate exposure 1) increases the abundance of apoptotic bodies in renal cell cultures, 2) promotes a rapid (within 15 min) redistribution of phosphatidylserine from the interior to the exterior of the cell membrane, 3) increases ceramide production. Evidence for oxalate-induced necrosis of renal cells includes the finding that 1) membrane integrity is lost, 2) cellular nuclei become swollen 3) DNA degradation is observed but "laddering" is not observed 4) cells fail to stain in the "TUNEL" assay. These changes in cell function appear to be due to oxalate-induced increases in oxidant stress in renal cells. Oxalate exposure increases free radical production in renal cells and antioxidants can reduce both the increase in free radical production and the increase in toxicity. Overexpression of bcl-2, an antioxidant gene that localizes to mitochondrial membranes, also reduces oxalate toxicity. Thus mitochondria may be the source of the increased free radical production that is observed after oxalate exposure. Recent data using isolated mitochondria support this possibility and provide evidence that oxalate may trigger mitochondrial changes via one or more lipid signals from the plasma membrane.



"Cytoprotection" and Calcium Oxalate Crystal Retention in the Kidney

J.C. Lieske

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Adhesion of urinary crystals to distal tubular cells could be a critical event that triggers a cascade of responses ending in kidney stone formation. Human and rat studies have suggested that tubular injury may play a role in crystal retention. For example, increased excretion of cellular enzymes has been observed in the urine of stone-forming humans and of oxalate-loaded rats, and it has been postulated that cellular damage could result from crystal deposition. However when Scheid, Menon, and colleagues administered the nephrotoxin gentamicin to rats together with oxalate, crystal deposition was enhanced, suggesting that cellular damage might precede and promote crystal retention, rather than being a consequence of the crystals.

We employed monolayer cultures of distal nephron-derived MDCKI cells as a model to study crystal-cell interactions. COM crystal adhesion progressively fell during the first 6 days after plating. The time-dependent fall in crystal binding was accelerated by prostaglandin (PG)E_2 supplementation, and delayed by blockade of PG production. Crystals avidly adhered to cells that migrated in to repair a scrape wound made in the monolayer, and both 4 hours and 3 days after a transient ischemic insult. Treatment of physically- or ischemically-injured cells with trypsin or neuraminidase reduced crystal binding to baseline levels, suggesting increased exposure of cell surface glycoproteins mediated the effect. Exposure of MDCKI cells to an oxalate challenge was also associated with increased crystal adhesion, as was exposure of cells to uric acid (UA) crystals. PGE_2 ablated the response to physical injury and ischemia. These studies provide new evidence that binding of COM crystals to renal cells is enhanced by injurious stimuli that modify exposure of cell surface crystal binding molecules, and that this response of injured cells is modulated by physiologic signals such as PGE_2. Subtle renal injury could be an important factor that promotes crystal adhesion along the nephron and favors calculus formation.

Our experiments in cultured renal cells, and those of Verkoelen and colleagues, suggest that cell surface crystal binding proteins are present on the surface of regenerating and migrating cells. Candidate molecules include heparan sulfate proteoglycan, since Iida and colleagues detected the mRNA encoding this glycoprotein in the kidneys of ethylene glycol-treated rats, and the protein was immunohistochemically localized to regions of the kidney containing crystalline deposits. Recently, a 110-kDa calcium-binding protein on the surface of continuous inner medullary collecting duct (cIMCD) cells was isolated by Kleinman and colleagues using hydroxyapatite chromatography, and it was postulated that it functions as a receptor for calcium crystals. This putative receptor was named nucleolin-related protein (NRP) because the amino acid sequence closely resembles rat nucleolin. Further studies will be necessary to define the availability and relative importance of NRP *in vivo* as a molecule that mediates adhesion of calcium crystals to the cell surface. Additional crystal receptor molecules on the surface of renal cells are being actively pursued, such as phosphatidylserine and hyaluronic acid.

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Our recent studies suggest that while diverse injurious stimuli increase adhesion of COM crystals to renal cells, the presence of exogenous PGE_2 appears to defend against this response. PGE_2 could exert this action by regulating expression or exposure of crystal binding molecules on the surface of regenerating cells. If tubular cells along the collecting duct *in vivo* respond similarly to these forms of stress, it is possible that subtle renal injury could increase the likelihood of crystal retention and eventual kidney stone formation, and intrarenal PGs could serve a protective function by preventing adhesion of crystals to cells undergoing repair or regeneration. Renal cellular responses to crystals include changes in gene expression and proliferation. Oxalate ion itself can also damage cells and/or induce changes in gene expression and proliferation. The net effect of signaling pathways initiated by calcium oxalate crystals and oxalate ion could be interstitial scarring, fibrosis and loss of renal function, especially in states of heavy crystalluria such as primary or secondary hyperoxaluria.

Selected Bibliography

1. Hammes MS, Lieske JC, Pawar S, Spargo BH, Toback FG: Calcium oxalate monohydrate crystals stimulate gene expression in renal epithelial cells. *Kidney Int.* 48:501-509, 1995
2. Lieske JC, Leonard R, Swift HS, Toback FG: Adhesion of calcium oxalate monohydrate crystals to anionic sites on the surface of renal epithelial cells. *Am. J. Physiol.* 270:F192-F199, 1996
3. Bigelow MW, Wiessner JH, Kleinman JG, Mandel NS: Surface exposure of phosphatidylserine increases calcium oxalate crystal attachment. *Am. J. Physiol.* 272:F55-F62, 1997
4. Thamiselvan S, Hackett RL, Khan SR: Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. *J. Urol.* 157:1059-1063, 1997
5. Verkoelen CF, van der Boom BG, Houtsmuller AB, Schröder FH, Romijn JC: Increased calcium oxalate monohydrate crystal binding to injured renal epithelial cells in culture. *Am. J. Physiol.* 274:F958-F965, 1998
6. Kleinman JG, Sorokina EA: Cloning and preliminary characterization of a calcium-binding protein closely related to nucleolin on the apical surface of inner medullary collecting duct cells. *J. Biol. Chem.* 274:27941-27946, 1999
7. Jonassen J, Cooney R, Kennington L, Gravel K, Honeyman T, Scheid C: Oxalate-induced changes in the viability and growth of human renal epithelial cells. *Journal of the American Society of Nephrology* 10:S446-S451, 1999
8. Lieske JC, Huang E, Toback FG: Regulation of renal epithelial cell affinity for calcium oxalate monohydrate crystals. *Am. J. Physiol.* 278:F130-F137, 2000 Recent Review
9. Lieske JC, Deganello S, Toback FG: Cell-crystal interactions and kidney stone formation. *Nephron* 81:S8-S17, 1999

The Role of Endocytosis: Lessons from the Lung

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How do epithelial cells in the respiratory tract respond to particles similar in size to calcium oxalate crystals? Particle – epithelial cell interactions are common because of breathing. We inhale approximately 20,000 liters or 20 cubic meters of air each day. Relatively clean cities in the eastern United States typically experience respirable particle concentrations ranging from 10 to 100 μg/m^3. Polluted cities around the world may experience a range approximately 10 times higher. Finally, some workplace activities such as welding may have respirable particle concentrations of the order of 5,000 μg /m^3. Typically, 10 to 30% of these inhaled particles deposit on respiratory tract surfaces.

The nature of epithelia in the respiratory tract varies. In the conducting airways, there are ciliated cells which are interspersed with secretory cells such as goblet cells and Clara cells. Together they constitute a mucocilliary transport system. Like the kidney, bulk flow can remove unattached particles from the organ. Thus, most particles landing on the airways are moved mouthward on a moving carpet of mucus. However, some particles are taken up through endocytic mechanisms. Increased particle uptake by epithelial cells is correlated with diminishing particle size or the presence of airway injury. In some cases, particles appear to be transported through epithelial cells. Particles may then ultimately be taken up by connective tissue macrophages in subjacent tissues.

The peripheral lung is covered with type 1 and type 2 epithelial cells. The type 1 cell forms the air blood barrier which is specialized for gas exchange. Epithelial cells cover a large surface area (>100m^2) and have a thickness less than one micron. In contrast, the type 2 cell is a cuboidal cell responsible for alveolar secretions, particularly surfactant phospholipids and apoproteins.

Most particles depositing on alveolar surfaces are removed by resident phagocytic cells, primarily alveolar macrophages. In some circumstances, neutrophils may also be recruited and they too may ingest particles or pathogens. Because ingestion is relatively rapid, few particles are taken up by respiratory epithelial cells. However, an epithelial pathway does exist and has been frequently documented. We have only observed epithelial cell endocytosis by type 1 cells; type 2 cells do not appear to participate in endocytosis. Like the airways, some particles are transported through epithelial cells. These particles move through the basement membrane and enter lymphatics. Some may appear in connective tissue or lymph node macrophages. Uptake by alveolar epithelial cells is more likely as particle load increases and as particle size decreases.

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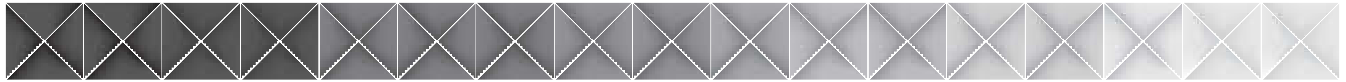
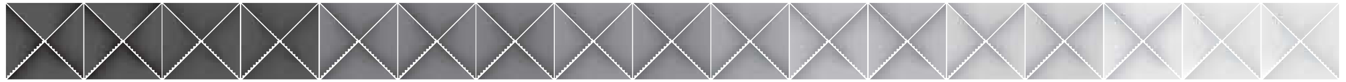


What are the consequences of particle uptake? Airway epithelial cells are not simply a passive barrier. Particularly in the airways, they have been shown to secrete a variety of mediators such as IL-1ß and EGR-1. Little is know about the cytokine responses of type 1 epithelial cells in the pulmonary parenchyma. Particles penetrating the epithelial barrier move along channels in the basement membrane. Particles are then usually taken up by connective tissue, macrophages, or lymph node macrophages.

We have used technologies which may be useful to scientists studying calcium oxalate stone disease. First, we have developed a variety of methods for examining particles and their anatomic location. Laser scanning confocal microscopy is a valuable tool for imaging particles in relation to their anatomic location. Simultaneously, one can look at physiologic changes in living cells such as redistribution of calcium. Another powerful technology is EELS (electron energy loss spectroscopy) l—a powerful technology for imaging low atomic number elements. Both calcium and oxygen can be visualized and quantified by this technique. In this way, calcium oxalate particles could be recognized by their elemental signature. Second, we have developed a technique called twisting cytomagnetometry. This strategy utilizes spherical homogeneous magnetic beads coated with specific ligands which in turn bind to specific receptors on epithelial cells. By magnetizing these particles and twisting them we can quantitatively measure the mechanical properties of epithelial cells. We have shown that airway epithelial cells become stiffer after exposure to a variety of mediators. These techniques could be applied to isolated renal epithelial cells as they respond to calcium oxalate particles.

These studies have been supported by NIH- ES00002.



Oxalate Interaction with Renal Epithelial Cells: Physiologic Adaptations or a Chain of Toxic Events?

H. Koul

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Urolithiasis is a multifactorial disorder and it is unlikely that a single factor would be responsible for the entire spectrum of this disorder. However, one important factor in the pathogenesis of this disorder is alterations in oxalate metabolism. While only a few patients have overt or classic hyperoxaluria, many more show a slight but definite increase in urinary oxalate. The manner by which oxalate promotes stone formation is not entirely clear. During the last decade, our laboratories have been actively involved in understanding effects of oxalate on renal epithelial cells. We and others have shown that renal epithelial cells do not perceive oxalate as an inert metabolite, but respond to oxalate exposure by displaying a program of events, including alterations in gene expression, reinitiation of DNA synthesis and apoptosis and or necrosis. We also observed that renal epithelial cells of proximal and distal origin respond differently to oxalate exposure and that oxalate exposure promotes COM-crystal binding to renal cells.

In this talk I will discuss these results in detail and try to raise arguments in favor of the possibility that much of these changes seen in response to oxalate exposure reflect physiological adaptations rather than a chain of toxic events.

Crystal Deposit Patterns in the Kidney: A Comparative Microscopic Analysis of Crystal Location and Composition in Renal Tubular Acidosis (RTA) and Non-RTA Patients

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Introduction: Crystals of calcium phosphate or calcium oxalate can be found in the urine of most individuals. On the other hand, urinary stones, of which 70-80% contain calcium oxalate (CaOx), afflict only a small percentage of the population. The process of renal crystal formation is poorly understood, and although studied extensively, there are several basic questions that remain unanswered including the initial site of crystal nucleation/growth and whether such formation is primarily intracellular, intraluminal, interstitial or a combination or two or more mechanisms. Furthermore, because the transit time from the collecting duct to the bladder is approximately ten minutes, there is insufficient time for any crystals to grow to the size necessary to cause symptoms. Finlayson, therefore, proposed in 1978 that no renal stones could form without a fixed point (i.e. nidus) in the collecting system or papilla. Prior to Finlayson, Randall described calcified subepithelial papillary plaques, which he theorized represented nidi for urinary stone formation. Higher incidence of Randall's plaques has been found in stone formers vs. patients undergoing endoscopy for non-stone disease (74% vs. 43%). The pathogenesis of Randall's plaques and their role in stone formation is yet to be elucidated. We have initiated a study designed to correlate the morphology and distribution of Randall's plaques in stone forming and non-stone forming patients, as well as the microscopic anatomy and chemical composition of crystalline material in these lesions, to the patient's clinical and metabolic abnormalities. In the present preliminary report, we have compared the findings in the uncommon renal tubular acidosis stone former to patients with more prevalent conditions.

Methods: Renal papillary biopsies from two patients with a history of multiple stone events (mixed calcium-oxalate (CaOx) & mixed hydroxyapatite), nephrocalcinosis and a 24-h urine consistent with RTA were compared to biopsies from 2 patients with a single stone event (CaOx) and one patient with a struvite stone. Tissue specimens underwent light microscopic, Fourier Transform-Infrared (FT-IR), and transmission electron microscopic analysis. Calcium salts were identified by light microscopy using Yasue stain while chemical composition of these deposits were analyzed on 10 micron thick sections by FT-IR at multiple sites within the interstitium and along the nephron.

Results: Two clearly distinct calcium deposit patterns were identified by both light microscopy and transmission electron microscopy in RTA and non-RTA patients. In the RTA patients, calcium deposits were found within tubular and urothelial cells throughout the biopsy specimen. FT-IR analysis revealed calcium phosphate, calcium carbonate but no CaOx. In

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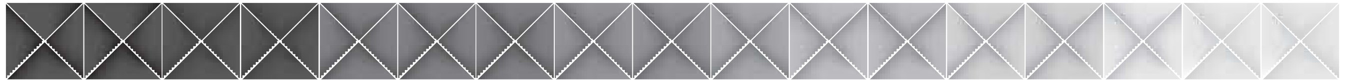

contrast, in the 3 non-RTA patients calcium deposits were extracellular in the interstitium surrounding all parts of the nephron and concentrated toward the papillary tip. FT-IR analysis revealed mostly calcium phosphate, calcium carbonate, and some CaOx. RTA patients had an extensive distribution of Randall's plaques in comparison to a moderate distribution in the non-RTA patients.

Conclusions: The findings in this preliminary report may confirm the observations of other studies showing a primary distribution of calcium phosphate crystal deposits in the interstitium of a diverse group of non-RTA patients. The different clinical and histological pattern noted in the RTA patients, in comparison to non-RTA patients, may suggest a unique cellular mechanism leading to stone formation in RTA patients. Lastly, further data are needed to determine which of the three alternative pathways for kidney stone formation and growth presented in the introduction applies to the various subtypes of stone formers.

Polymorphism-Mutation Synergism in AGT*

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Primary hyperoxaluria type 1 (PH1) is the best studied of the monogenic calcium oxalate kidney stone diseases. It is caused by a functional deficiency of the liver-specific, peroxisomal, pyridoxal phosphate-dependent enzyme alanine:glyoxylate aminotransferase (AGT). Numerous mutations and polymorphisms in the gene encoding AGT have been identified, but in only a few cases has the causal relationship between genotype and phenotype actually been demonstrated. In order to rectify this situation, we have determined the effects of the most common naturally-occurring amino acid substitutions (both normal polymorphisms and disease-causing mutations) on the properties, especially specific catalytic activity, of purified recombinant His-tagged AGT.

Our results showed the following: 1) normal human His-tagged AGT can be expressed at high levels in *E. coli* and purified in a correctly folded, dimerized and catalytically-active state; 2) presence of the common Pro11Æ Leu polymorphism decreases the specific activity of purified recombinant AGT by a factor of three; 3) AGTs containing four of the most common PH1-specific mutations (i.e. Gly41Æ Arg, Phe152Æ Ile, Gly170Æ Arg, Ile244Æ Thr) are all soluble and catalytically active in the absence of the Pro11Æ Leu polymorphism, but in its presence all lead to protein destabilization and aggregation into inclusion bodies; 4) naturally-occurring and artificial amino acid substitutions that lead to peroxisome-to-mitochondrion AGT mistargeting in mammalian cells also lead to destabilization and aggregation in *E. coli;* 5) the PH1-specific Gly82Æ Glu mutation abolishes AGT catalytic activity by interfering with pyridoxal phosphate binding, as does the artificial Lys209Æ Arg mutation at the putative site of cofactor Shiff-base formation.

Not only do these studies demonstrate the causal relationship between five of the most common mutations found in PH1 and their enzymic phenotypes, but also they demonstrate the remarkable functional synergism between four of these mutations and the common Pro11Æ Leu polymorphism. In addition, our results suggest that this polymorphism alone, which varies in allelic frequency from 2% to 30% in different racial groups, might not be without consequences for the general population.

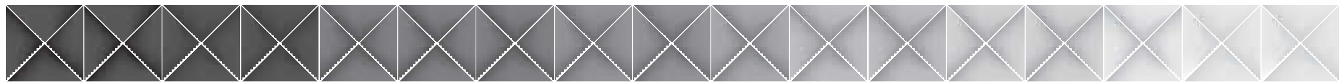
Bibliography:

Barratt, T.M. & Danpure, C.J. (1999) Hyperoxaluria. In *"Pediatric Nephrology"*, 4th edition, Williams & Wilkins, Baltimore, eds. Barratt, T.M., Avner, E.D. & Harmon, W.E. Chapter 36, pp 609-624.

Danpure, C.J. (2000) Genetic disorders and urolithiasis. *Urol. Clin. North Am.*, 27, 287-299.

Danpure, C.J. (2000) Primary hyperoxaluria. In *"The Metabolic and Molecular Bases of Inherited Disease"*, 8th edition, McGraw-Hill, New York, eds. Scriver, C.R., Beaudet, A.L., Sly, W.S. & Valle, D., in press.

*Part of this work has recently been published, as follows: Lumb, M.J. & Danpure, C.J. (2000) Functional synergism between the most common polymorphism in human alanine:glyoxylate aminotransferase and four of the most common disease-causing mutations. *J. Biol. Chem.* in press. (see also http://www.jbc.org – Papers in Press, Aug 25).



AGXT Gene Mutations and Their Influence on Clinical Heterogeneity of Type 1 Primary Hyperoxaluria in Italian Patients

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Background: Primary hyperoxaluria type 1 (PH1) is an autosomal recessive disorder caused by a deficiency of alanine-glyoxylate aminotransferase (AGT), which is encoded by a single copy gene (AGXT). Molecular diagnosis was used in conjunction with clinical, biochemical and enzymological data in order to evaluate genotype-phenotype relationships.

Methods: Twenty unrelated PH1 Italian patients were classified into three groups according to their clinical presentation: group A (most severe form of PH1); group B (an adult form) and group C (mild to moderate decrease in renal function). They were analyzed using the single strand conformation polymorphism (SSCP) technique, followed by the sequencing of abnormal mobility bands of the 11 AGXT exons.

Results: Both the mutant alleles were identified in 19 out of 20 patients. Thirteen different mutations in exons 1, 2, 4 and 10 were recognized. Normalized AGT activity was lower in group A than in group B ($p<0.01$). Double heterozygous patients presented with an earlier age at onset of the disease (p:0.045) and were more frequent in group A (75%) than in the other 2 groups (8%, p:0.0044). Mutation T444C was more frequent in the severe form (p:0.0098) while the opposite was observed for G630A (p:0.014). Homozygotes for the G630A mutation showed a higher AGT residual activity (p:0.0001).

Conclusions: This study confirms the presence of allelic heterogeneity of the AGXT which may partly account for the phenotypic heterogeneity in PH1. Homozygous genotypes were more frequent than expected and were associated with a less severe form of the disease.

Molecular Genetics of Primary Hyperoxaluria Type 2

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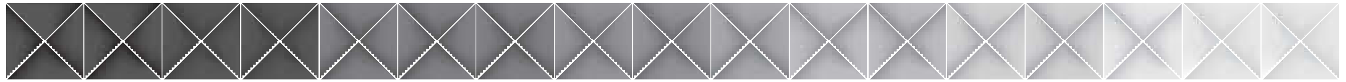
Primary hyperoxaluria type 2 (PH2) is a rare monogenic disease characterized by excessive urinary oxalate and L-glycerate excretion. The severity of clinical complications in PH2 patients can range from asymptomatic to end-stage renal failure due to massive deposits of calcium oxalate crystals in the kidney. The disease is a result of the absence of an enzyme with glyoxylate reductase and hydroxypyruvate reductase activities (GRHPR). Recent breakthroughs have occurred in our understanding of the molecular basis of PH2. In this presentation I will briefly discuss the molecular genetics of PH2. The real breakthrough in our understanding of the molecular biology of PH2 has come through the public database of expressed sequence tags, or EST's. We use the human EST database to identify a full-length clone that homology to known plant and bacterial GRHPR enzymes. We demonstrated that this cDNA encodes a functional enzyme with GR, HPR, and D-glycerate dehydrogenase (DGDH) activities. GRHPR EST's are found in essentially every human EST library available, suggesting that the GRHPR gene is expressed in all, or nearly all human tissues. The implications of this ubiquitous expression on treatment options for PH2 will be discussed.

We next used the cDNA sequence to design PCR primers to walk along the genomic DNA and map the genomic structure of the human GRHPR gene. The gene encompasses approximately 9 kb, containing 9 exons, on chromosome 9. Just remember, number 9! Single-Strand Conformation Polymorphism (SSCP) assays were developed to identify mutations in the coding regions of the GRHPR gene from PH2 patients. We have genotyped 11 PH2 patients to date. All patients have inactivating mutations in the GRHPR gene. Six different mutations have been identified. These mutations include deletion mutations that result in a frameshift or disruption of a splicesite, nonsense mutations, and missense mutations. Ten of the 11 patients we have genotyped are homozygous for one of the six mutations identified to date. Due to this high proportion of homozygotes, we used microsatellite markers in close linkage with the GRHPR gene to investigate the possibility that the patients are the offspring of related individuals. Our data suggest that two thirds of our patients are the offspring of either closely or distantly related persons. Furthermore, genotyping revealed the possible presence of a founder effect for the two most common mutations and the location of the gene telomeric to marker D9S1874.



Primary Hyperoxaluria Type 2: Biochemical and Genetic Characterisation of the Disease

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Primary hyperoxaluria type 2 (PH2) is caused by a deficiency of glyoxylate reductase and hydroxypyruvate reductase activities. We have recently cloned and expressed the gene for human glyoxylate/hydroxypyruvate reductase (GRHPR) which has enabled further characterisation of the protein and investigation of the underlying genetic basis of PH2. Although GRHPR mRNA is present in all tissues tested, the expression and catalytic and immunoreactivity is greatest in liver where it is in close proximity to other enzymes involved in glyoxylate metabolism. Kinetic studies on the purified enzyme show that it has a higher affinity (i.e. lower Km) for hydroxypyruvate than glyoxylate in the presence of NADPH. However, glyoxylate reductase activity should be used for the diagnosis of PH2 as other enzymes (notably LDH) are able to reduce hydroxypyruvate. Diagnosis of PH2 based on deficient glyoxylate reductase activity and negative immunoreactivity identified one patient without L-glycericaciduria. Thus the absence of this urinary metabolite should no longer be used to exclude PH2.

Analysis of GRHPR RNA from patients with PH2 has identified a number of mutations including defective splicing in which part of intron 4 is retained.

Chloride Channel Dysfunction and Hypercalciuria in Dent's Disease

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Dent's disease, or X-linked recessive nephrolithiasis, is primarily a proximal tubulopathy. Its hallmark feature is low molecular weight (LMW) proteinuria, almost always present in extreme degrees. But hypercalciuria is also common; the majority of affected males have renal parenchymal calcification and half have stone disease. Stones are usually calcium oxalate (but occasionally calcium phosphate, even though nearly all patients can acidify the urine normally). Hypercalciuria explains the risk of stones in these patients; urinary excretion of citrate and oxalate are normal. The hypercalciuria is largely diet-dependent, and serum levels of 1,25(OH) vitamin D tend to be mildly elevated. PTH levels are usually normal or even low.

The gene encoding a voltage-gated chloride channel (ClC-5) was identified through positional cloning and is inactivated by mutation in patients with Dent's disease. As of today, a total of 55 distinct mutations have been identified in 75 unrelated families from across the world. These include 15 missense, 13 nonsense, 1 in-frame codon insertion, six splice-site, 15 frameshift, and 5 microdeletion mutations. There is no correlation between nature of mutation and severity of phenotype. Most of the 15 missense mutations are in conserved transmembrane domains, but several occur within intracellular tails or loops of the protein and could inactivate channel function through disrupting trafficking, regulation, or interaction with other proteins.

The ClC-5 channel is expressed in the proximal tubule, medullary thick ascending limb (mTAL) of Henle's loop, and a-intercalated cells of the collecting duct. Its role in these distal nephron segments is for now poorly understood, but in the proximal tubule we know that ClC-5 is expressed in subapical endosomes, where it colocalizes with the proton-ATPase responsible for acidification of the endosomal lumen. Chloride entry into these endosomes serves to dissipate the positive charge generated by the proton pump, and allows maximal acidification necessary for processing of adsorbed proteins. Defective endosomal function explains the failure to reabsorb LMW proteins. It has been speculated that endosomal dysfunction could alter recycling of membrane transporters and thereby explain the defects in carrier-mediated transport. Lumenal parathyroid hormone (PTH), a LMW protein, may participate in the pathophysiology of Dent's disease. PTH is found in great excess in the urine of patients with Dent's disease. Failure to reabsorb filtered PTH in the early proximal tubule could result in activation of apical PTH receptors in more distal segments. Apical PTH receptors have recently been reported to be coupled to internalization of sodium-phosphate cotransporters (NaPi2) in proximal tubule, and the vitamin D 1a-hydroxylase has recently been shown to be present not only in proximal tubule but also in distal nephron.

Continued

Two mouse models of ClC-5 inactivation reproduce the hypercalciuria of Dent's disease. In transgenic mice with an antisense ribozyme targeted against ClC-5, expression of the chloride channel is reduced. These mice have hypercalciuria that corrects with dietary calcium restriction. Mice with targeted disruption of the ClC-5 gene lack the channel completely, and exhibit not only hypercalciuria but also LMW proteinuria , aminoaciduria, glycosuria, nephrocalcinosis, and bone deformities, reproducing much of the phenotype of the human disease. Use of such models offers promise of a fuller understanding of the pathophysiologic consequences of inactivation of the ClC-5 chloride channel.

References:

Lloyd SE, Pearce SHS, Fisher SE, et al: A common molecular basis for three inherited kidney stone diseases. Nature. 379:445-449, 1996

Scheinman SJ: X-linked hypercalciuric nephrolithiasis: Clinical syndromes and chloride channel mutations. Kidney Int. 53:3-17, 1998

Luyckx VA, Leclercq B, Dowland LK, Yu ASL: Diet-dependent hypercalciuria in transgenic mice with reduced CLC5 chloride channel expression. Proceedings of the National Academy of Sciences 21: 12174-12179, 1999

Norden AGW, Lapsley, M, Thakker RV, et al: The tubular proteinuria of Dent's disease (CLCN5 mutation) comprises proteins in the mass range from insulin to intact immunoglobulin G and provides a new approach to estimation of in vivo glomerular sieving coefficients. Journal of the American Society of Nephrology 11:93A (abstract), 2000

Wang SS, Devuyst O, Wang XT, et al: Generation and characterization of a new Dent's disease mouse model lacking the ClC5 chloride channel. Journal of the American Society of Nephrology 11:2096A (abstract), 2000

Primary Hyperoxaluria Type 1 (PH1) in Switzerland: Unusual Clinical and Biological Aspects

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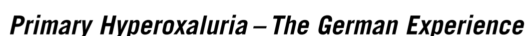
The birth prevalence of PH1 for Switzerland was recently reported by our group to be 1 per 10^5 live births. However, the prevalence is likely to be underestimated as PH1 may be overlooked. Diagnostic problems occur if clinical symptoms and signs are mild or even absent, or if manifestation is atypical. In addition, the classification of pyridoxine responsiveness may be difficult. We report 4 unusual PH1 patients.

Diagnosis of PH1 was made in two non-related children during family screening because of PH1 in affected siblings: one had urolithiasis and one infantile oxalosis. *Patient 1* had persistent hyperoxaluria; *patient 2* with borderline hyperoxaluria was finally diagnosed based on the homozygous G630A mutation. Both children, now aged 10 and 14 years, had neither clinical symptoms nor nephrocalcinosis. *Patient 1* has refused therapy; patient 2 is partially pyridoxine responsive. *Patient 3* presented with urolithiasis, moderate hyperoxaluria (0.5 – 1.0 mmol/1.73 m^2, normal < 0.5) and additional hypercalciuria (8 mg/kg; normal < 4). Keeping in line with this finding was the stone analysis revealing a mixture of calcium oxalate – monohydrate (90%) and – dihydrate (10%). Diagnosis of PH1 was proven by liver biopsy.

Primary non-responsiveness to pyridoxine was demonstrated in *patient 4* whose brother had died of infantile oxalosis. High doses of pyridoxine (30-50 mg/kg per day), started at the age of 3 weeks and given for 2 months, had no effect on urinary oxalate excretion. Mean urinary oxalate/creatinine ratios (mmol/mol) in weekly samples were 1360 off medication and 1490 on pyridoxine. A second trial with pyridoxine (6 mg/kg per day) at 14 years of age, prompted by the demonstration of a heterozygous G630A mutation, resulted in an immediate reduction of the urinary oxalate by 64% to nearly normal values. The absent response shortly after birth remains unexplained as non-compliance was unlikely and systemic oxalosis was not present.

Conclusions:

1. Borderline hyperoxaluria or absence of urolithiasis/nephrocalcinosis do not preclude diagnosis of PH 1.
2. Prevalence rates for PH1 are likely to be underestimated.
3. PH1 may be overlooked if hypercalciuria coexists.
4. Response to pyridoxine shortly after birth may be different than in older children, hence a further trial is recommended.



Primary Hyperoxaluria – The German Experience

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Primary hyperoxaluria (PH) is a heterogeneous disease with high variations of age at first symptom, of progression into renal failure and subsequent systemic oxalosis. Early diagnosis is mandatory but is often delayed or overlooked and therefore it appears, as if PH is easily underestimated. Data from Europe (UK, Switzerland and France) suggest that 1 in 60,000 to 120,000 children suffers from the primary form of PH. The disease is, however, far more common in other countries like Tunisia, where PH1 is the cause of ESRF in 13% of pediatric patients as compared to only 0.3% in Europe. As clinical papers from different countries give contrasting results, and as the disease is so very heterogenous, it is of importance to collect data from different regions to better elucidate its metabolic and molecular genetic background. We therefore collected data on patients with primary hyperoxaluria (types 1 and 2) in a first nationwide survey in Germany in 1994. Then, 36 patients were included into the survey, with 32 of them being younger than 18 years. In six families more than one child was affected. Mean age of first symptom was low with 2.6 years, it, however showed a wide range (0.1 – 13 years). Most of the patients came to medical attention because of either urolithiasis or nephrocalcinosis, 4 children were only diagnosed in renal failure. The diagnostic approach included the determination of urinary oxalate in 31 (86 %), of glycolate in 20 (56 %) and of L-glyceric acid in 2 (5.6 %) patients. Liver biopsy was performed in 18 patients, expressing a defect or mistargeting of alanine:glyoxylate aminotransferase. All these procedures assured diagnosis of either PH1 (n=27) or PH2 (n=2) in 81% of patients. As the rest of the patients also showed a typical course of the disease, it is now to speculate, whether they have a currently still "unclassified" third form of primary hyperoxaluria.

The clinical status in 1994 showed 22 children without the necessity of renal replacement therapy, two of them got a pre-emptive liver transplant. From the 10 patients with end-stage renal failure (ESRD), one received a single kidney transplant, 3 got a liver transplant, and combined liver-kidney transplantation was performed in 5 children. Half of the patients with ESRD died, 4 of them although they received a transplant. We concluded at this time, that the prevalence of PH is 1:400,000 births in Germany, thus being higher than expected. Prognosis was still poor although transplantation was offered as early as possible.

In 2000 we started a second survey to get an update on incidence, diagnostic and therapeutic procedures and on follow up of the patients included in our first questionnaire. Currently, data are still collected and will be presented at the NIH oxalosis workshop. In addition the German data will be compared with data obtained in a first nationwide survey performed in 1997/98 in the United States.

Primary Hyperoxaluria: Strategies in Clinical Management

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Patients with GFR >30 mL/min/1.73 m2: Reduction of stone forming activity and preservation of renal function are primary goals. Fundamental is an increase in oral fluid intake to maintain urine oxalate concentration as low as possible, preferably <0.3 mmol/L. Pyridoxine is effective in reducing urine oxalate excretion rates in approximately 20-30% of patients with type I primary hyperoxaluria. While there is agreement that a pyridoxine trial is indicated in all patients with primary hyperoxaluria type I, the dose of pyridoxine, the duration of an adequate trial, and a consistent definition of response have not been well defined. In our practice, 3 of 19 PHI patients showed complete pyridoxine responsiveness (urine oxalate <0.5 mmol/24 hrs). Four additional patients had reduction in urine oxalate excretion rates of ≥50% to 0.5-1.1 mmol/24 hours. The remaining 12 patients (63%) showed no benefit from pyridoxine at a mean dose of 5.3 mg/kg/day for three to six months.

Neutral phosphate therapy reduces calcium oxalate crystalluria and supersaturation and results in improved inhibitory activity of the urine for calcium oxalate crystal formation. Long-term results of neutral phosphate treatment have been reported. Citrate therapy also reduces calcium oxalate supersaturation in the urine. However, there is limited information available regarding long-term treatment trials with citrate in primary hyperoxaluria. Information comparing the efficacy of citrate and neutral phosphate is not available. Preliminary results in four of our patients suggest a greater reduction in calcium oxalate supersaturation and more inhibition of calcium oxalate crystal formation in the urine after neutral phosphate therapy when compared with citrate. Confirmation of these results in a larger number of patients is needed.

Our studies of six children with PHI demonstrated no significant reduction in urine oxalate excretion rates with a low oxalate diet (20 mg/m2/day) as compared with an unrestricted oxalate diet. After an oral oxalate load of 50 mg/m2, there was no significant increase in urine oxalate excretion. Given the difficulty and importance in achieving compliance with consistently high oral fluid intake, added dietary restrictions are more likely to be deleterious to compliance than to be of therapeutic benefit.

With a program of high oral fluid intake, neutral phosphate therapy, and regular patient reassessment, stone formation can be controlled in most patients. In 12 patients with PHI and 8 patients with PHII, treated contemporaneously, the median rate of change in GFR in PHI was -1.65 mL/year and in PHII -1.04 mL/year. Our patients with PHII showed less metabolic stone formation and better preservation of renal function than those with PHI.

Recent work has focused attention on injury to the renal epithelial cell and interstitium induced by calcium oxalate monohydrate crystals. Calcium oxalate crystals induce expression of PDGF mRNA in renal tissue (Hammes, 1995). In an ethylene glycol model of hyperoxaluria development of interstitial fibrosis was reduced by addition of enalapril (Toblii, 1999). Clinical studies are needed to evaluate effects of ACEI in patients with primary hyperoxaluria.

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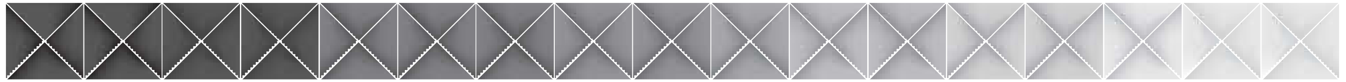
Reduced and Declining GFR: Goals are preservation of renal function and to minimize time of ESRD. Plasma oxalate values, in our experience, remain favorable at <20umol/L (oxalate oxidase assay, nl 0.2-3.0 µg/L) in patients with primary hyperoxaluria who have a GFR of >25. As GFR declines, plasma oxalate begins to rise. Work of Hoppe and Worcester suggests that plasma supersaturation for calcium oxalate occurs at a plasma oxalate concentration of 30-48. In patients with declining renal function, serum creatinine and plasma oxalate should be monitored frequently. Attention to appropriate modification of citrate and/or phosphate therapy are needed. Early initiation of dialysis at a glomerular filtration rate of <20 or plasma oxalate of >20-30 µmol/L, or transplantation at an equivalent time, are indicated.

End Stage Renal Disease Management: Goals are maximum oxalate removal and minimum time on dialysis until transplantation can be accomplished. Patients with PH may have markedly elevated predialysis plasma oxalate, ranging from 40-160 umol/L. Dialysis oxalate clearance for hemodialysis, CVVHDF, slow nocturnal dialysis, CAPD, and CCPD have been studied. Hemodialysis and slow nocturnal dialysis provide the highest clearance. Oxalate removal should match the amount of daily oxalate production. In many such patients, no single dialysis modality is sufficient. Daily dialysis and/or combined modalities (such as CCPD plus five days weekly hemodialysis) may be needed to achieve adequate oxalate removal. Predialysis plasma oxalate <30 µmol/L and oxalate removal of >2 mmol/day are desirable.

Transplantation: Transplantation should be performed as early as feasible after the GFR has declined to <20-30 mL/min/1.73 m2. With both kidney alone and combined kidney/liver transplantation, plasma oxalate concentration declines promptly after transplantation. In our patients, mean plasma oxalate at 30 days post transplantation was 10.3 and 10.4 µmol/L, respectively. More time (as long as 5 years or more) is required for urine oxalate excretion rates to decline in patients who receive combined kidney/liver transplantation. Those with aggressive pretransplant dialytic intervention show much earlier declines in urine oxalate values. Until the urine oxalate normalizes, the allograft is at risk of oxalate injury. In patients with functioning renal allografts, the same treatment modalities used in patients with well preserved renal function are indicated.

Kidney only transplantation should be considered in type II PH, patients who are fully pyridoxine responsive, and patients who will have more than six months wait for orthotopic liver transplant. Factors favoring combined kidney/liver transplantation are systemic oxalosis, a previously failed renal allograft because of oxalate deposition, and a very large daily oxalate production rate. Living donor liver transplant, liver before kidney transplant, and preemptive liver transplantation require critical evaluation.



Pyridoxine Sensitivity in Primary Hyperoxaluria Type 1 (PH1)

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The coenzyme of the alanine:glyoxylate aminotransferase is pyridoxal phosphate. For many years it has been known that pyridoxine may reduce or even normalize oxalate excretion in 25-30% of patients with primary hyperoxaluria type 1. Usually pharmacological doses have to be appplied. In other PH1 subjects, supplementation of a small dose suffices. The remaining PH patients do not have a measurable reduction in hyperoxaluria.

A review of the literature and results of a survey among experts are given.

The following procedure outlines a consensus of these experts in order to test B6 sensitivity: After repeated urinary measurements, B6 is started at 5 mg/kg b.i.d and subsequently increased to 20 mg/kg (always <1000 mg/d), if no normalization of the excretion occurs. Consequently, full (normal excretion), partial (>30% reduction of oxalate excretion, but still abnormal test result) and no response (<30% reduction and pathological test result) are defined.

Effective Oxalate Removal

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Background and Aims: Because oxalate is quite exclusively excreted by the kidney, renal failure induces chronic oxalate retention, which represents a threat for relentless deposition of calcium oxalate crystals in body tissues. Despite some evidence indicating that renal failure may increase both intestinal secretion and bacterial catabolism of oxalate, the majority of newly generated oxalate must be removed by renal replacement therapy (RRT), including hemodialysis (HD) or peritoneal dialysis (PD).

Therefore, dialysis schedules should be tailored in order to achieve a zero oxalate balance, or at least, to set plasma oxalate at levels compatible with undersaturation with respect to calcium oxalate. Aim of this presentation is to report results of our studies on dialysis kinetics and removal rates of oxalate in patients with end-stage renal failure (ESRF) due to either primary hyperoxaluria (PH) or oxalosis-unrelated renal diseases.

Methods and Material: Oxalate kinetics were assessed on RRT patients undergoing both HD or PD. HD patients were given either standard bicarbonate dialysis (BD) or hemodiafiltration (HDF), using high-flux, high-efficiency cartridges. PD patients were administered different schedules, including continuous ambulatory (CAPD) or cyclic continuous (CCPD) peritoneal dialysis. Studies have been performed on patients with both oxalosis-unrelated and PH-related ESRF. Some of these were re-studied after pyridoxine supplementation.

Plasma, urine and dialyzate were assayed for urea, creatinine, oxalate (ion-chromatography) and glycolate (HPLC) by techniques previously described and widely experimented in our Institution. Renal and dialysis clearances were calculated by using standard formulas, which account for by residual diuresis and whole dialysate collections.

Oxalate balance was calculated from the difference between estimated oxalate appearance rate (OxAR) and dialysis removal. In this context, the contribution of intestinal excretion to oxalate balance was assumed as negligible. State of saturation of plasma ultrafiltrate with respect to calcium oxalate monohydrate (ßCaOx) was calculated by an ab-initio calculation performed by our own computer system, with values given in a scale whereby 1 is saturation, > 1 supersaturation.

Results: *Oxalosis-Unrelated Patients:* Dialysis clearances of oxalate in HD patients were mildly higher with HDF than standard BD (113±45 vs 82 ± 25 ml/min, p=0.04). However, since mean dialysis time was shorter on HDF, overall removal of oxalate was similar during both procedures (657±144 vs 698±151 µmol/sesssion, on HDF and BD, respectively). These schedules produced plasma levels of oxalate quite always below 50 µmol/L, which, in our calculations, represents the upper limit of plasma oxalate yielding undersaturation at usual levels of serum ultrafilterable

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calcium. In fact, mean ßCaOx was 1.0±0.3 in samples taken at the end of the longest inter-dialytic interval, and remained well below saturation in all other samples. Mean estimated OxAR matched quite closely dialysis removal.

Patients on PD behaved similarly, in that peritoneal clearances of oxalate were 5.8±4.9 and 8.3±3.1 ml/min in CAPD and CCPD, respectively, fixing mean plasma levels at 30±15 µmol/L and ßCaOx at 0.57±0.31. A one-month course of pyridoxine supplementation (75 and 300 mg/daily) had no effect on oxalate plasma levels in 9 PD patients.

PH-related patients: Despite larger dialysis removal of oxalate (1780±397 µmoles/session), plasma levels were hugely elevated in these patients, yielding mean pre-dialysis plasma oxalate of 170±21 µmol/L, with ßCaOx of 3.4±0.6. Blood sampled during the inter-dialytic interval was virtually always supersaturated with calcium oxalate. Kinetic studies showed that the maximum increase in plasma oxalate and ßCaOx was reached early, 6 to 12 hours after the end of dialysis session, leveling off thereafter. We have taken this peculiar pattern of the oxalate over time curve as indicating deposition of newly generated oxalate outside its miscible pool, a feature that was not shared by oxalosis-unrelated HD patients. From many other determinations on PH patients on RRT, we confirm that body fluids are unable to maintain in solution oxalate at ßCaOx exceeding 3 to 4. This simple procedure also permits to roughly assess the amount of newly generated oxalate that deposits in body tissues.

Conclusion: From these results we conclude that current dialysis techniques, both HD and PD, allow to obtain effective removal of oxalate in the majority of the patients with oxalosis-unrelated ESRF. The finding that, in these patients, bony content of oxalate was only slightly increased and unrelated to duration of RRT, is in agreement with this contention. Conversely, the same procedures are largely inadequate to achieve oxalate balance, unless dialysis time or dialysis clearances are four to five fold increased as compared to oxalosis-unrelated patients. This produces progressive accumulation of oxalate as is confirmed by serial measurements of bony oxalate. The burden of oxalate accumulated during dialysis time has a potentially negative impact in view of these patients being considered for any transplantation strategies. It seems therefore useful to assess amounts of newly produced and accumulated oxalate, to increase as much as possible dialysis efficiency, and explore possible responsivity to pyridoxine.

Nutritional Aspects of Oxalate

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Oxalate is consumed in normal human diets as a component of nuts, fruits, vegetables, grains, and legumes. Urinary oxalate, derived from a combination of exogenous (dietary) and endogenously synthesized oxalate, is a primary determinant of the level of calcium oxalate saturation. Changes in urinary oxalate exert a greater influence on the risk of calcium oxalate stone formation than do proportional changes in urinary calcium.

Dietary factors which lead to an increase in urinary oxalate will lead to a corresponding increase in risk for formation of calcium oxalate-containing stones. Three key precipitating factors are high oxalate consumption, hyperabsorption of dietary oxalate, and increased endogenous oxalate production. It is now recognized that dietary oxalate can make a greater contribution to total urinary oxalate excretion than was previously assumed. With daily oxalate intakes approaching 250 mg, an amount approximately equivalent to that contained in a 1.5 oz serving of cooked spinach, absorbed oxalate may contribute up to 50% of the total urinary oxalate.

A summary of important factors which may affect the contributions of endogenous oxalate synthesis and dietary oxalate to urinary oxalate is as follows:

Endogenous oxalate synthesis

• Ascorbate?

• Protein/amino acids?

Dietary oxalate

• Amount ingested

• Bioavailability

-Propensity of Ca and Mg to decrease absorption

-Macronutrient composition?

-Presence and concentration of oxalate-degrading bacteria ?

-Transit times through the G.I. tract?

There are still a number of key questions which must be answered in order to increase understanding of these complex relationships. Starting with endogenous synthesis, it has not been clearly established whether high-dose ascorbate supplementation leads to clinically significant increases in urinary oxalate. The more germane question appears to be whether calcium oxalate stoneformers are more susceptible to ascorbate-induced increases in urinary oxalate, and within this subpopulation, are hyperoxaluric stoneformers more susceptible than normooxaluric stoneformers? Another key question is whether high protein intakes lead to increased endogenous oxalate synthesis and/or increased risk for urolithiasis. Increasing dietary protein has been reported to increase urinary oxalate in some but not all studies; specific effects on endogenous synthesis have not been reported in the literature. However, regardless of the significance of the relationship between dietary protein and endogenous oxalate synthesis, high protein intakes may increase risk via the documented adverse effects on urinary calcium, uric acid, and citrate levels.

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With regard to dietary oxalate contribution to urinary oxalate, it has recently been established that although percent oxalate absorption decreases with increasing oxalate doses, higher oxalate intakes lead to significantly higher levels of urinary oxalate (Holmes et al. 2000). In comparison to non-stoneformers, idiopathic calcium oxalate stoneformers have been demonstrated to exhibit a higher oxalate absorption in some but not all studies. Whether there is a subgroup of these individuals who exhibit a higher endogenous oxalate synthesis remains to be established.

Oxalate bioavailability must also be considered. Although increased dietary calcium, as well as magnesium, have been clearly demonstrated to reduce oxalate absorption and urinary oxalate, how important is timing of ingestion in this regard? The report of associations between higher calcium intakes and reduced risk of stone formation, and between supplemental calcium (presumed to be taken between meals or in conjunction with low-oxalate meals) and increased risk of stone formation within a large cohort of older women (Curhan et al. 1997) suggests that dietary calcium and oxalate must be ingested together in order to reap the benefit of a calcium-induced inhibition of oxalate absorption. It has been demonstrated that when oxalate and calcium are ingested together, the greatest calcium-induced depression of oxalate absorption occurs between 2 and 6 hours postingestion, which coincides with the time at which both oxalate and calcium would be expected to have reached the small intestine (Liebman and Costa, 2000).

Other key questions related to the issue of oxalate bioavailability include the effects of macronutrient composition (e.g., dietary fat content), presence and concentration of gastrointestinal oxalate-degrading bacteria, and transit times through the gastrointestinal tract. Ultimately, the following “bottom-line” questions must be answered in order to fully understand the role of dietary modifications in prevention of calcium oxalate kidney stones:

Are there specific dietary modifications which can be adopted by high-risk patients (e.g., reducing amount and/or bioavailability of dietary oxalate) which will lead to clinically significant reductions in urinary oxalate?

Will dietary-induced reductions in urinary oxalate lead to clinically significant reductions in kidney stone risk?

References:

Curhan et al. Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting risk for kidney stones in women. *Ann Intern Med* 1997;126:497-504.

Holmes et al. The contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int* 2000 (in press).

Liebman and Costa. Effects of calcium and magnesium on urinary oxalate excretion after oxalate loads. *J Urol* 2000; 163:1565-9.