HOW ELEVATED OXALATE CAN PROMOTE KIDNEY STONE DISEASE: CHANGES AT THE SURFACE AND IN THE CYTOSOL OF RENAL CELLS THAT PROMOTE CRYSTAL ADHERENCE AND GROWTH

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1. ABSTRACT

The present review assesses the mechanisms by which oxalate-induced alterations in renal cell function may promote stone disease focusing on 1) changes in membrane surface properties that promote the attachment of nascent crystals and 2) changes in the expression/secretion of urinary macromolecules that alter the kinetics of crystal nucleation, agglomeration and growth. The general role of renal cellular injury in promoting these responses and the specific role of urinary oxalate in producing injury is emphasized, and the signaling pathways that lead to the observed changes in cell surface properties and in the viability and growth of renal cells are discussed. Particular attention is paid to evidence linking oxalate-induced activation of cytosolic phospholipase A2 to changes in gene expression and to the activation of a second signaling pathway involving ceramide. The effects of the lipid signals, arachidonic acid, lysophosphatidylcholine and ceramide, on mitochondrial function are considered in some detail since many of the actions of oxalate appear to be secondary to increased production of reactive oxygen molecules within these organelles. Data from these studies and from a variety of other studies *in vitro* and *in vivo* were used to construct a model that illustrates possible mechanisms by which an increase in urinary oxalate levels leads to an increase in kidney stone formation. Further studies will be required to assess the validity of various aspects of this proposed model and to determine effective strategies for countering these responses in stone-forming individuals.

2. INTRODUCTION

Discussions of the various factors that contribute to stone disease generally fall into two categories: 1) those focusing on factors within the urine that modulate the rate of crystal nucleation, agglomeration and growth, and 2) those focusing on cellular surface properties that promote the adherence and/or uptake of nascent crystals by renal epithelial cells. Such arbitrary divisions make it simpler to approach the large literature related to kidney stone disease. However, it is important to recognize that the kidney is a complex organ comprising multiple cell types that are subjected to wide variations in ionic/osmotic conditions, in urinary flow rates and in local and circulating hormone levels. Moreover, stone disease encompasses a family of conditions/disorders that culminate in the deposition of kidney stones within the kidneys of affected individuals. These disorders reflect a variety of genetic alterations (and a variety of biochemical/metabolic perturbations) that can be exacerbated or ameliorated by environmental factors,

Table 1. Macromolecules Affecting Crystal Formation

Macromolecule	References
Glycosaminoglycans (GAGs)	4-7
Tamm-Horsfall glycoprotein	8-10
Nephrocalcin	4, 11-13
Uropontin (Osteopontin)	14-16
Urinary prothrombin fragment 1	17
Inter-α-trypsin inhibitor (bikunin light chain)	18-21
Calgranulin	22
Fibronectin	23
α-1-microglobulin	24
β-2-microglobulin	25
Human serum albumin	3,26

including diet, climate, lifestyles, etc. (1, 2). Thus no single review can adequately convey the complexity of this disease process. That being said, the present review will attempt to bridge these two large areas of interest to stone researchers by providing some insight as to the mechanisms by which renal cells can modify urine composition (and hence modify the kinetics for crystal nucleation, agglomeration and growth). This review will also discuss current evidence as to the types of cellular alterations that enhance crystal adherence and uptake.

3. FORMATION AND GROWTH OF KIDNEY STONES

3.1. Crystal Nucleation

Crystal nucleation reflects a phase transformation in which a solid phase forms from a supersaturated solution (3). Urine is normally supersaturated with respect to calcium, phosphate and oxalate such that conditions are favorable for crystal formation. However, urine contains numerous macromolecular components, including many secreted by renal epithelial cells (3, 4-26, and listed in table 1), that normally create a meta-stable equilibrium that limits crystal formation (27-29). Given the dynamics of this equilibrium, small changes in ionic composition can lead to spontaneous crystal formation in the absence (homogeneous nucleation) of exogenous particulates (30). In the presence of foreign materials (cell debris, bacteria, seed crystals, etc), nucleation, in this case heterogeneous nucleation, occurs much faster (31-34).

3.2. Crystal Growth

Crystal growth, defined as the rate of deposition of ions onto a crystal nidus, occurs at the interface between the liquid and solid phases and involves both solute transfer and interfacial processes (3). Growth of crystals is influenced by the rate of diffusion of various solutes and by properties of the crystal surface, including surface charge. The latter can be altered by the adsorption of various urinary constituents (table 1). Moreover, analysis of extracts of kidney stones confirms that these urinary macromolecules are indeed incorporated into the crystal lattices of stones (35-38). These considerations suggest that the kinetics of crystal growth *in vivo* likely differ from those *in vitro* when growth occurs in pure solutions of inorganic chemicals. Moreover, the kinetics *in vivo* may

differ in different segments of the nephron since urine concentrations and composition vary in different parts of the nephron (39, 40) and since different macromolecules are produced in different regions. For example, crystals formed in the early portion of the nephron may be coated with nephrocalcin, which is produced only in proximal tubules and in the thick ascending limb of the loop of Henle (11), or with inter-alpha-inhibitor (bikunin), found in both proximal and distal tubules (7, 19). Crystals forming later in the nephron may interact with Tamm-Horsfall glycoprotein (8), urinary prothrombin fragment 1 (4, 17) and/or osteopontin (4, 5), which are produced in the thick ascending limb of the loop of Henle and/or in the distal convoluted tubules. Perhaps of more importance is the possibility that the sites of production of these macromolecules and/or the characteristics of the secreted proteins may be altered in stone disease. Studies in experimental stone-forming animals (19, 42-44) and in human kidney stone patients (45, 46) have supported this possibility. Nonetheless, it is not clear whether such changes are a cause or a consequence of the disease process, since 'stone-forming conditions' *in vitro* (e g. exposure to elevated oxalate levels or to calcium oxalate crystals) have been shown to increase the expression of many of these compounds (see below and 47).

3.3. Agglomeration

Agglomeration, or aggregation of particulate materials, may also play a major role in the progression of kidney stone disease (39, 40, 48, 49). In simple solutions, crystal agglomeration is affected by particle density, by the relative affinities between particles and/or by the affinities of a particle for a particular surface. In more complex solutions (such as urine), crystal agglomeration is also affected by various biological materials, including secreted proteins and cellular debris, which can adsorb onto the surfaces of crystals and increase the probability of agglomeration, even at low levels of supersaturation (49). The agglomeration/aggregation process can occur quickly, producing large particles within the lumen of kidney tubules or on the surfaces of tubular cells. When the latter occurs, the particle is then fixed at a single site within the nephron where it may grow to form a stone. A similar sequence can occur when particles agglomerate onto membrane fragments that later bind to the surface of other cells within the nephron (29, 31).

4. CELLULAR RESPONSES TO OXALATE EXPOSURE

4.1 . Crystal Attachment

A number of studies have demonstrated that renal epithelial cell injury promotes crystal attachment as a consequence of changes in the surface properties of affected cells and/or unmasking of attachment sites beneath or between cells. Studies by Mandel and his collaborators (50-54) were among the first to demonstrate this linkage, showing an increase in crystal binding following treatments that disrupt tight junctions, allowing crystal access to membrane constituents normally restricted to basolateral membranes (50). Related studies suggested that the crystal attachment may also involve extracellular matrix proteins and/or cellular binding sites for these proteins, which are normally masked in intact monolayers. For example,

studies by Lieske and colleagues (55, 56) demonstrated an attenuation of crystal attachment in BSC-1 cells by treatment with arginine-glycine-aspartic acid-serine (RGDS, a tetrapeptide that bind to integrins), or by pretreatment with fibronectin, a connective tissue protein containing this peptide sequence. Similarly, Verkoelen *et al.* (57) demonstrated that crystal attachment to wounded MDCK monolayers could be attenuated by enzymatic removal of hyaluronic acid (6). The latter is a structural component of connective tissue that is also expressed by subconfluent cultures of renal epithelial cells *in vitro* and by damaged renal epithelial cells *in vivo* (58). Other studies demonstrated that renal cell injury, produced by exposure to elevated oxalate levels (59-64), also leads to increase in the attachment of crystals (53, 65) which can subsequently be internalized and deposited in interstitial spaces and/or remain at the cell surfaces serving as *nidi* for further crystal growth. Such processes provide fixed sites where crystals may ultimately grow to form stones (the 'fixed particle' theory of stone formation, see 66, 67).

Other studies indicated that redistribution of the phospholipid, phosphatidylserine (PS), to the surface of the cell can promote crystal binding. This phospholipid is normally restricted to the inner leaflet of the membrane via an ATP-dependent process that may involve the actin cytoskeleton (68). When cells are damaged, membrane PS redistributes to the surfaces of cells, where it can serve as a recognition signal for engulfment and removal by macrophages (69). Interestingly, studies using artificial membranes showed that crystal nucleation and growth readily occurs on phosphatidylserine liposomes (70). Similarly, studies using intact cells demonstrated that conditions leading to an increase in the PS content of renal cell membranes (54), or producing a redistribution of phosphatidylserine (PS) from the interior to the exterior of the cell membrane (52, 65) promote crystal attachment to the renal cells. This damage-induced increase in crystal attachment can be blocked by pretreatment with annexin V (52, 65), a protein that selectively binds to superficial PS and that may limit further access to these sites (69).

Other surface molecules also contribute to the adherence of crystals. Studies by Lieske and coworkers (55, 56) and by Verkoelen *et al.* (71) have implicated sialic acid-containing glycoproteins in crystal attachment, demonstrating reduced crystal binding following neuraminidase treatment. These charged proteins might also promote nucleation of calcium oxalate dihydrate crystals onto the surface of renal cells by determining the crystal face that selectively interacts with the cell surface (72). Other investigators suggested an involvement of collagen (73) and of a molecule resembling nucleolin in crystal binding (74). Thus it would seem that an array of secreted and surface-associated molecules is involved in crystal attachment to cells, and that various pathological conditions can alter the availability and/or orientation of these molecules at the cell surface. Furthermore, since crystal size is several orders of magnitude greater than the dimensions of these individual molecules, it is likely that crystals may attach to many molecules or perhaps to several adjacent cells. In this regard, our own work (60)

and that of others (53) noted that renal toxins (including oxalate at high concentrations) produce foci or 'strings' of damaged cells that also serve as foci for crystal attachment (65). The precise manner by which cell death propagates between cells is as yet unknown.

4.2. Crystal Uptake

A number of studies have suggested that calcium oxalate crystals can be taken up by kidney cells. In particular, both *in vivo* (75) and *in vitro* studies (55, 73, 76-78) provided evidence that the adherence of crystals may activate endocytic pathways that bring about an internalization of attached crystals. This uptake involves an active engulfment of surface particles, following attachment to specific sites on the cell surface. Once crystals are internalized, they may dissolve within lysosomes or be released at the basolateral surface, a process that may account for the appearance of crystals in the renal interstitium in experimental stone disease (75). Crystal endocytosis can be enhanced by treatments that stimulate cell migration and/or proliferation, such as ADP and cytokines (55) and inhibited by agents that interact with cell adhesion sites including RGDS, fibronectin and heparin (55, 73, 77). Crystal endocytosis is also attenuated by treatments that inhibit protein kinase C or that disrupt the cytoskeleton (78).

4.3. Proliferation

The process of crystal endocytosis (and/or events at the membrane surface that enhance crystal attachment) triggers a number of other physiological processes including those regulating cellular proliferation and those regulating apoptotic cell death. Proliferative responses have been observed in a number of cell types following exposure to various calcium-containing crystals. For example, in synovial cells, exposure to crystals of hydroxyapatite or calcium phosphate stimulated mitosis (79, 80). Similarly in renal epithelial cells, exposure to oxalate in its crystalline (73, 76, 77, 81) or in its soluble form (59, 82, 83) increased cellular proliferation, as evidenced by increased expression of a number of early response genes (c-jun, c-myc, Egr-1 nur 77), an increase in the incorporation of thymidine into DNA, and an increase in cell number (see 47 for a recent review). This proliferative response could serve to promote further crystal attachment as the dividing cells round up and detach, unmasking new attachment sites on the underlying basement membrane (76).

4.4. Activation of Phospholipase A2

The exact sequence of events involved in crystal-induced proliferation remains unclear. However, both the process of endocytosis and the processes regulating membrane phospholipid asymmetry involve the actin filament lattice, which is intimately linked to a number of membrane bound signaling molecules, including tyrosine kinases, phospholipases, phosphoinositide kinases, etc. (reviewed in 68). These signaling molecules control multiple pathways within cells including those regulating the activity of cytosolic phospholipase A2 (cPLA2), an enzyme that hydrolyzes the acyl group from the sn-2 position of phospholipids. This enzyme produces a number of active byproducts including arachidonic acid and

Table 2. Oxalate-induced changes in gene expression that may influence crystal growth or deposition

Gene	Model system	Function/ potential role in stone disease	References
osteopontin (uropontin)	rat stone models, renal cell culture	stone matrix, inhibitor of crystallization	41, 95-100
Tamm Horsfall protein	rat stone models	inhibitor of oxalate crystallization, attachment	9, 99, 100
clusterin	renal cell cultures	renal injury marker	101
bikunin	rat stone models, renal cell culture	Kunitz-type protease inhibitor; may block crystal attachment	7, 19, 100, 102
Gla-1: matrix γ carboxyglutamic acid containing protein	rat stone models	Inhibitor of mineralization or stone formation	98, 100
osteonectin (secreted protein, acidic, rich in cysteine-SPARC)	rat stone models	remodeling, healing, interacts with ECM	98, 100
heparan sulfate proteoglycan	rat stone models	Inhibitor of crystallization	7, 44, 100
PDGF-A, connective tissue growth factor; alpha-1 and alpha-2 -collagens,	renal cell cultures; rat stone models	growth factors, ECM. remodeling	81, 100
plasminogen activator inhibitor	renal cell cultures	ECM accumulation/ remodeling	81

assorted lysophospholipids that can, in turn, stimulate other signaling pathways within cells (84). Activation of PLA2 has been observed in a number of pathologies involving injury of renal epithelial cells (85-87). In addition, patients with active stone disease show elevations in plasma arachidonate levels and in the arachidonate content of red cell membranes (88, 89), suggesting a role for PLA2 in the etiology of stone disease. Thus, it was of interest to discover that cPLA2 is also activated in renal epithelial cells following exposure to elevated concentrations of oxalate (90, 91). Studies on MDCK cells demonstrated that oxalate produces a time- and concentration-dependent increase in the activity of cPLA2 (90) that may be responsible for many of the other cellular actions of oxalate. Indeed activation of this enzyme may be responsible for the induction of a number of immediate early genes that are involved in cellular proliferation, since inhibition of PLA2 activity blocked the oxalate-induced increases in Egr-1, c-jun and c-myc mRNAs (91). Whether PLA2 activation is secondary to oxalate-induced changes in membrane asymmetry (65) or to other actions on the cell membrane is, as yet, unknown.

The induction of several early response genes appears to be mediated by specific lysophospholipid byproducts of PLA2 activation, since lysophosphatidylcholine (Lyso-PC), but not arachidonic acid or lysophosphatidic acid, mimicked the effects of oxalate on gene expression (91). Lysophospholipids released by PLA2 activation have also been implicated in the proliferative responses of OK cells (92), vascular smooth muscle cells (93) and mouse renal proximal tubular cells (94).

4.5. Induction of Proteins Regulating Crystal Binding
In addition to changes in gene expression that lead to proliferation, exposure to oxalate or to calcium oxalate crystals leads to increased expression of genes encoding a variety of secreted proteins that have been shown to alter the rate of crystal nucleation and growth (7, 9, 19, 42-44, 81, 95-102), as summarized in table 2, and in more detail in a recent review (47). Although the full sequence of events promoting oxalate-induced changes in

gene expression are not yet fully understood, it is likely that generation of lipid signaling molecules by PLA2 activation plays a role in this process. As indicated above, Lyso-PC mimics the stimulatory effects of oxalate on the expression of several immediate early genes in MDCK cells (91). Moreover, Lyso-PC modulates gene expression in a variety of other cells. For example, Lyso-PC induces expression of adhesion proteins and proteins known to elicit remodeling of the extracellular matrix, including vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 (103-105). Lyso-PC also stimulates expression of growth factors including PDGF-A and PDGF-B (104), and heparin binding epidermal-like growth factor (106). In rat aortic muscle cells (107), (but not in human cultured endothelial cells; 104), Lyso-PC increases the expression of monocyte chemoattractant protein-1 (MCP-1). MCP-1 is induced in renal cells following exposure to oxalate ions or to calcium oxalate crystals (108) and its expression has been associated with inflammatory responses in a variety of kidney diseases (109) including perhaps, the inflammation produced by crystal deposition in stone disease (108, 110). In a variety of cells, Lyso-PC has been shown to stimulate numerous transcription pathways, including AP-1 (111-113), SP-1 (114), Egr-1 (115) and NF-kappa beta (116); thus, in addition to direct effects on gene transcription, Lyso-PC may exert secondary effects on gene expression that are regulated via these transcription factors. While the roles of Lyso-PC and other potential mediators of oxalate-induced changes in gene expression remain incompletely understood, it is clear that altered synthesis and release of a variety of proteins can have profound consequences for crystallization events within the kidney. Oxalate-induced synthesis of specific kidney proteins may represent an important adaptive response that could be a potential target for therapies to limit stone disease.

4.6. Activation of Neutral Sphingomyelinase
Renal cell exposure to oxalate also increases the cellular levels of ceramide, another lipid signaling molecule (117, 118). Oxalate-induced increases in ceramide are of interest because in other cells, ceramide has been shown to mediate a variety of responses, including proliferation, differentiation, cytotoxicity and death (119). Intracellular

concentrations of ceramide can increase through a variety of pathways (119) but it is likely that in renal cells, activation of neutral sphingomyelinase plays an important role (118).

The nature of the stimulus for neutral sphingomyelinase activation in response to oxalate exposure is not completely understood. Oxalate may exert a direct effect, but it is also likely that oxalate works indirectly, via PLA2 activation in renal epithelial cells, since pretreating MDCK cells with AACOCF3, a selective inhibitor of cytosolic PLA2, blocked the oxalate-induced increases in ceramide production (118). Moreover, arachidonic acid, a lipid signal generated by PLA2 activation, mimics oxalate actions on ceramide production in renal cells (118). A similar link between the PLA2 and the ceramide pathways also occurs in HL-60 human leukemia cells (120). Several studies in neural cells have suggested that the cross-talk between these two pathways may also occur in the opposite direction, namely, ceramide may enhance the activation of PLA2 (121, 122). This possibility has not yet been confirmed in renal epithelial cells.

Irrespective of the trigger(s) eliciting ceramide production, it is clear that increased availability of this lipid can exert multiple effects on cellular function, including apoptosis (119, 123). Several studies have linked oxalate toxicity to apoptotic renal cell death, as described in more detail below (117, 124, 125), although the precise links between ceramide and renal cell death have not yet been completely elucidated.

4.7. Mitochondrial Actions of Lipid Mediators

Two lines of evidence support the notion that alterations in mitochondrial function are responsible for many of the effects of oxalate (and by extension for the effects of its lipid mediators). First, many studies (reviewed in 126) indicate that mitochondria are the major source of oxidants in mammalian cells. Second, in whole kidney and in isolated kidney cells there is evidence supporting an increase in oxidant stress following oxalate exposure. Early evidence came from studies on experimental animals which found that experimental increase in urinary oxalate loads also increased the excretion of lipid peroxides (127-130) and decreased the levels/activities of renal antioxidant enzymes (131, 132). Interpretation of such studies was somewhat difficult, however, given the long time course required to observe these changes (weeks to months), by the multitude of cell types within the kidney and by myriad confounding factors (diet, hormonal status, etc.) that can affect experimental outcomes. To determine whether oxalate exposure could elicit a direct, acute oxidant stress in renal cells, a number of studies have used monolayer cultures of renal epithelial cells to identify oxalate-induced changes in mitochondrial production of reactive oxygen molecules. Many studies from our laboratory and others have observed that oxalate exposure in a variety of kidney cell lines increases free radical production (assessed using redox sensitive dyes - see 59, 62, 133) and induces toxicity (59-64, 83, 117, 134, 135). In other studies, oxalate treatment of renal cell cultures increased production of lipid peroxides and increased the release of intracellular enzymes (64, 135). Moreover, oxalate-induced toxicity and free radical production could

be attenuated by pretreatment with various antioxidants (60-62, 83, 134-136), by treatments that disrupt electron transport in mitochondria (133), and by genetic manipulations that enhance expression of bcl-2 (117), a protein that modulates mitochondrial permeability (137).

Recent studies have examined mitochondrial responses to oxalate and its lipid mediators in more detail. Treatment of renal epithelial cells with oxalate, ceramide, arachidonic acid or lysophosphatidylcholine evoked a number of changes in mitochondrial function, including depolarization of the mitochondrial membrane (47, 138). The changes in mitochondrial membrane potential were accompanied by an increase in the production of reactive oxygen molecules in isolated mitochondria, by an increase in the oxidation of mitochondrial thiols and by an increase in the peroxidation of mitochondrial membranes (133, 138). These findings provide support for earlier studies in which oxalate exposure was shown to increase the permeability of the inner mitochondrial membrane (139) and to increase the oxidation of mitochondrial glutathione (131). Interestingly, the effects on mitochondrial membrane potential could be blocked by AACOCF3, an inhibitor of cPLA2 (138). Thus, it seems likely that the increase in renal oxidant stress caused by exposure to high levels of oxalate in vitro (62, 134, 135) or in vivo (64, 127-130, 132) are due to changes in mitochondrial function mediated by lipid signaling molecules.

4.8. Apoptotic Cell Death

Perturbations in mitochondrial function are often accompanied by an increase in mitochondrial permeability and a release of pro-apoptotic factors. These factors in turn trigger the activation of cellular caspases, serine proteases that have been linked to apoptotic cell death (126, 140, 141). Exposure to high levels of oxalate *in vitro* (117, 124) and *in vivo* (125) leads to an increase in the abundance of apoptotic (and necrotic) renal epithelial cells by a process involving increased oxidant stress (83, 117). Our recent findings provide a possible explanation for these findings, e.g. oxalate actions at the cell membrane generate lipid signals that act on mitochondria to elicit an increase in oxidant stress and an increase in apoptotic death. The increase in oxidant stress also constitutes a positive feedback loop for the generation of ceramide, since pretreatment with antioxidants can block oxalate induced increases in ceramide (118). Antioxidant treatments also block the induction of a number of immediate early genes (83) as well as oxalate-induced cell death (62, 134, 135), suggesting a role for oxidant stress in these responses. However, antioxidants failed to block oxalate actions on PLA2 activity (90), suggesting that activation of PLA2 may be proximal to, and perhaps responsible for, the mitochondrial alterations that produce an increase in oxidant stress in renal cells.

4.9. Apoptotic and Necrotic Cell Death Promote Crystal Nucleation and Attachment

As mentioned above, damaged cells exhibit membrane alterations that can promote adherence of crystals to the cell surface. In addition, damaged cells can foster crystal growth and deposition in another way, by providing cellular debris for crystal nucleation. Crystals
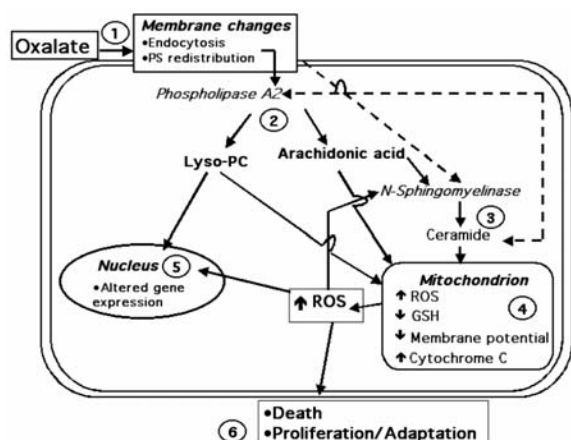
Figure 1. Oxalate-induced responses in renal cells. (1) Oxalate interacts with renal epithelial cells, leading to membrane changes that include endocytosis of crystals and redistribution of phosphatidyl serine (PS). (2) Among the cellular responses to oxalate exposure is an increased phospholipase A2 (PLA2) activity. PLA2 generates two lipid signaling molecules, arachidonic acid and lysophosphatidylcholine (Lyso-PC), which elicit a variety of intracellular effects. (3) Oxalate exposure also stimulates formation of ceramide, in part mediated via the lipid products of PLA2 activation. Oxalate may also exert a direct activation of neutral sphingomyelinase, and ceramide may activate PLA2, although the mechanisms for these pathways are not known, as indicated by the broken lines. (4) At the level of the mitochondria, lipid signaling molecules can act directly to promote generation of reactive oxygen species (ROS), to deplete reduced glutathione (GSH), to decrease mitochondrial membrane potential and to increase cytochrome C release. ROS may also trigger increased ceramide formation which could reinforce the mitochondrial changes. (5) Changes in gene expression may be triggered by increased ROS and/or increased Lyso-PC. Induced genes include immediate early genes and a variety of proteins that may be involved in crystal binding. (6) The net response to oxalate exposure can be cell death or alternatively, cellular adaptation, which may involve cellular proliferation and secretion of proteins that limit crystal growth or attachment.

formed *in vivo* all contain an organic core with a composition similar to that found in cellular membranes (4, 38, 142), and the addition of cellular membranes to artificial urine has been shown to increase crystal formation *in vitro* (31). Therefore, cellular damage that leads to shedding of dead cells and the generation of cellular debris within the tubular lumen would foster crystal nucleation. It would also foster the growth of crystals via agglomeration.

Figure 1 provides a model depicting the renal cellular responses to oxalate exposure that have been described in Section 4 of this review. In this model, oxalate exposure is linked to the generation of lipid signaling molecules, alterations in mitochondrial function and changes in gene expression. The net response in a population of renal cells may range from cell death to adaptation; the latter response may include cell

proliferation and the synthesis and secretion of proteins that influence subsequent cellular responses to oxalate.

5. SUMMARY AND PERSPECTIVES

The forgoing considerations suggest that there is considerable interplay between the renal cell and its environment, and suggest that this interplay can greatly affect the probability of stone formation. Agents and conditions that alter the surface properties of renal cells and/or that alter the secretion of urinary macromolecules promote stone formation. The scheme shown in figure 2 demonstrates how exposure to elevated oxalate levels can initiate this process. Oxalate exposure can elicit a redistribution of phosphatidylserine (PS) to the exterior leaflet of cell membranes, and, with longer exposure, produce a loss of membrane integrity (and death) in renal cells. Oxalate exposure can also alter the production and secretion of specific proteins and increase crystal access to sialic acid-containing or hyaluronic acid-containing glycoproteins at the cell surface. The net effect of these changes is an increase in the nucleation and attachment of crystals, a process that is facilitated by the presence of cellular debris, another byproduct of oxalate toxicity. Adherent crystals may be endocytosed by kidney cells and deposited at the basolateral surface of the cell, fostering interstitial crystal growth, or they may serve as a nidus for crystal agglomeration, fostering luminal crystal growth. Both pathways can eventuate in kidney stone formation.

While the available data are consistent with this model for stone disease, most of the *in vitro* data on renal cells utilized oxalate concentrations ranging from 0.250 mM to 1 mM, somewhat higher than would normally occur *in vivo*, making extrapolations from cellular data to human data somewhat difficult. [Note, however, that estimates of 'normal' urinary oxalate concentrations (~0.30 mM), which are derived from 24 hour samples, do not take into account normal diurnal variations in oxalate which can increase the concentration by 2 fold, for inter-nephron heterogeneity in oxalate secretion, or for acute increases in oxalate following a meal (another 1.5 fold increase) - see 67 for further review]. Similarly, most of the *in vivo* data employed relatively high oxalate loads in the experimental animals such that the results described above may only pertain to patients with relatively high levels of hyperoxaluria. Consistent with this conclusion is the observation that careful pathological examination of human kidneys with idiopathic calcium oxalate stone disease have not provided clearcut evidence of oxalate toxicity (143). Thus in human stone disease elevations in urinary oxalate may represent only one factor that can predispose toward the formation of kidney stones. Further studies will be required to determine how this factor interacts with other factors (diet, climate, genetics, exposure to other renal toxins, etc.) that have been shown to play a role in the etiology of stone disease.

6. ACKNOWLEDGEMENTS
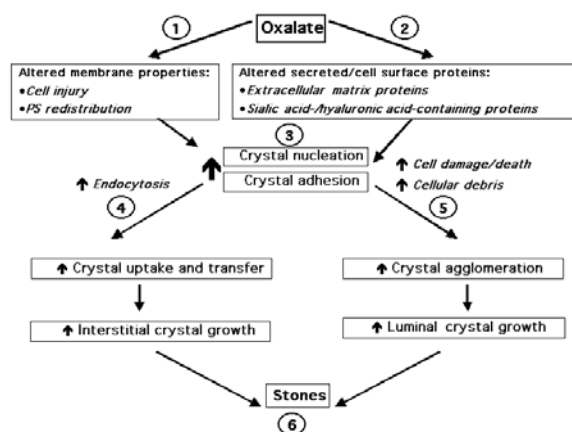
Figure 2. Proposed pathways to stone formation in renal epithelial cells. (1) Exposure to oxalate elicits changes in renal plasma membranes that can lead to cell injury and to the redistribution of membrane phosphatidyl serine (PS). (2) Oxalate exposure also leads to an alteration in secreted or cell surface proteins that may expose sialic acid-containing or hyaluronic acid-containing glycoproteins which can serve as oxalate-binding sites. In addition, oxalate exposure may induce synthesis and secretion of extracellular matrix proteins and other potential crystal binding proteins. (3) Changes in membrane lipids and proteins enhance the nucleation and adhesion of crystals to cells. (4) Adherent crystals may be endocytosed into kidney cells, where they may either be dissolved in lysosomes or released at the basolateral surface of the cell where they form sites of interstitial crystal growth. (5) Damaged cells or cellular debris may also form nidi for crystal agglomeration and luminal crystal growth. (6) Both pathways can lead to kidney stone formation.

7. REFERENCES

1. Goodman H. O, R. Brommage, D. G. Assimos & R. P. Holmes: Genes in idiopathic calcium oxalate stone disease. *World J Urol* 15, 186-194 (1997)

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

3. Wu W, D. E. Gerard & G. H. Nancollas: Nucleation at surfaces: the importance of interfacial energy. *J Am Soc Nephrol 10* Suppl 14, S355-S358 (1999)

4. Ryall R. L: Glycosaminoglycans, proteins, and stone formation: adult themes and child's play. *Ped Nephrol* 10, 656-666 (1996)

5. Umekawa T, M. Iguchi, E. Konya, T. Yamate, N. Amasaki & T. Kurita: Localization and inhibitory activity of alpha(2)HS-glycoprotein in the kidney. *Urol Res* 27, 315-318 (1999)

6. Verkoelen C. F, B. G. Van Der Boom & J. C. Romijn: Identification of hyaluronan as a crystal-binding molecule at the surface of migrating and proliferating MDCK cells. *Kid Int* 58, 1045-1054 (2000)

7. Eguchi Y, M. Inoue, S. Iida, K. Matsuoka & S. Noda: Heparan sulfate (HS)/heparan sulfate proteoglycan (HSPG) and bikunin are up-regulated during calcium oxalate nephrolithiasis in rat kidney. *Kurume Med J* 49, 99-107 (2002)

8. Hess B: Tamm-Horsfall glycoprotein and calcium nephrolithiasis. *Mineral Elect Metab* 20, 393-398 (1994)

9. Gokhale J. A, P. A. Glenton & S. R. Khan: Characterization of Tamm-Horsfall protein in a rat nephrolithiasis model. *J Urol* 166, 1492-1497 (2001)

10. Kokot F. & J. Dulawa: Tamm-Horsfall protein updated. *Nephron* 85, 97-102 (2000)

11. Nakagawa Y, M. Ahmed, S. L. Hall, S. Deganello & F.L. Coe: Isolation from human calcium oxalate renal stones of nephrocalcin, a glycoprotein inhibitor of calcium oxalate crystal growth. Evidence that nephrocalcin from patients with calcium oxalate nephrolithiasis is deficient in g-carboxyglutamic acid. *J Clin Invest* 79, 1782-1787 (1987)

12. Nakagawa Y: Properties and function of nephrocalcin: mechanism of kidney stone inhibition or promotion. *Keio J Med* 46, 1-9 (1997)

13. Wesson J. A, E. M. Worcester, J. H. Wiessner, N. S. Mandel & J.G. Kleinman: Control of calcium oxalate crystal structure and cell adherence by urinary macromolecules. *Kidney Int* 53, 952-957 (1998)

14. Hoyer J. R: Uropontin in urinary calcium stone formation. *Mineral Elect Metab* 20, 385-392 (1994)

15. Wesson J. A, R. J. Johnson, M. Mazzali, A. M. Beshensky, S. Stietz, C. Giachelli, L. Liaw, C. E. Alpers, W. G. Couser, J. G. Kleinman & J. Hughes: Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. *J Am Soc Nephrol* 14, 139-147 (2003)

16. Worcester E. M. & A. M. Beshensky: Osteopontin inhibits nucleation of calcium oxalate crystals. *Ann NY Acad Sci* 760, 375-377 (1995)

17. Grover P. K, S. C. Dogra, B. P. Davidson, A. M. Stapleton & R. L. Ryall: The prothrombin gene is expressed in the rat kidney: implications for urolithiasis research. *Eur J Biochem* 267, 61-67 (2000)

18. Atmani F, F. J. Opalko & S. R. Khan: Association of urinary macromolecules with calcium oxalate crystals induced in vitro in normal human and rat urine. *Urol Res* 24, 45- 50 (1996)

19. Atmani F, P. A. Glenton & S. R. Khan: Role of inter-alpha-inhibitor and its related proteins in experimentally induced calcium oxalate urolithiasis. Localization of proteins and expression of bikunin gene in the rat kidney. *Urol Res* 27, 63-67 (1999)

20. Médétognon-Benissan J, S. Tardivel, C. Hennequin, M. Daudon, T. Drueke & B. Lacour: Inhibitory effect of bikunin on calcium oxalate crystallization in vitro and urinary bikunin decrease in renal stone formers. *Urol Res* 27, 69-75 (1999)

21. Dean C, J. Kanellos, H. Pham, M. Gomes, A. Oates, P. Grover & R. Ryall: Effects of inter-alpha-inhibitor and several of its derivatives on calcium oxalate crystallization in vitro. *Clin Sci* 98, 471-480 (2000)

22. Pillay S. N, J. R. Asplin & F. L. Coe: Evidence that calgranulin is produced by kidney cells and is an inhibitor of calcium oxalate crystallization. *Am J Physiol* 275, F255-F261 (1998)

23. Tsujihata M, O. Miyake, K. Yoshimura, K. I. Kakimoto, S. Takahara & A. Okuyama: Fibronectin as a potent inhibitor of calcium oxalate urolithiasis. *J Urol* 164, 1718-1723 (2000)

24. Tardivel S, J. Medetognon, C. Randoux, M. Kebede, T. Drueke, M. Daudon, C. Hennequin & B. Lacour: Alpha-1-microglobulin inhibitory effect on calcium oxalate crystallization in vitro and decreased urinary concentration in calcium oxalate stone formers. *Urol Res* 27, 243-249 (1999)

25. Resnick M. I, D. Munday & W. H. Boyce: Urinary beta 2-microglobulin excretion and renal lithiasis. *Invest Urol* 18, 137-138 (1980)

26. Cerini C, S. Geider, B. Dussol, C. Hennequin, M. Daudon, S. Veesler, S. Nitsche, R. Boistelle, P. Berthezene, P. Dupuy, A. Vazi, Y. Berland, J. C. Dagorn & J. M. Verdier: Nucleation of calcium oxalate crystals by albumin: involvement in the prevention of stone formation. *Kidney Int* 55, 1776-1786 (1999)

27. Lanzalaco A. C, R. P. Singh, S. A. Smesko, G. H. Nancollas, G. Sufrin, M. Binette & J. P. Binette: The influence of urinary macromolecules on calcium oxalate monohydrate crystal growth. *J Urol* 139, 190-195 (1988)

28. Worcester E. M: Inhibitors of stone formation. *Semin Nephrol* 16, 474-486 (1996)

29. Coe F. L. & J. H. Parks: Does a failure of crystal inhibition cause calcium renal stones? *Nephron* 90, 361-364 (2002)

30. Nancollas G. H, S. A. Smesko, A. A. Campbell, C. F. Richardson, M. Johnsson, R. A. Iadiccico, J. P. Binette & M. Binette,: Physical chemical studies of calcium oxalate crystallization. *Am J Kidney Dis* 17, 392-395 (1991)

31. Khan S. R, P. N. Shevock & R. L. Hackett: Acute hyperoxaluria, renal injury and calcium oxalate urolithiasis. *J Urol* 147, 226-230. (1992)

32. Khan S. R & R. L. Hackett: Role of organic matrix in urinary stone formation: an ultrastructural study of crystal matrix interface of calcium oxalate monohydrate stones. *J Urol* 150, 239-245 (1993)

33. Khan S. R, S. A. Maslamani, F. Atmani, P. A. Glenton, F. J. Opalko, S. Thamilselvan & C. Hammett-Stabler: Membranes and their constituents as promoters of calcium oxalate crystal formation in human urine. *Calcif Tissue Int* 66, 90-96 (2000)

34. Wu W, H. Zhuang & G. H. Nancollas: Heterogeneous nucleation of calcium phosphates on solid surfaces in aqueous solution. *J Biomed Mater Res* 35, 93-99 (1997)

35. McKee M. D, A. Nanci & S. R. Khan: Ultrastructural immunodetection of osteopontin and osteocalcin as major matrix components of renal calculi. *J Bone Miner Res* 10, 1913-1929 (1995)

36. Atmani F, P. A. Glenton & S. R. Khan: Identification of proteins extracted from calcium oxalate and calcium phosphate crystals induced in the urine of healthy and stone forming subjects. *Urol Res* 26, 201-207 (1998)

37. Tawada T, K. Fujita, T. Sakakura, T. Shibutani, T. Nagata, M. Iguchi & K. Kohri: Distribution of osteopontin and calprotectin as matrix protein in calcium-containing stone. *Urol Res* 27, 238-242 (1999)

38. Ryall R, D.E. Fleming, P. K. Grover, M. Chauvet, C.J. Dean & V.R. Marshall: The whole truth: intracrystalline proteins and calcium oxalate kidney stones. *Molec Urol* 4, 391-402 (2000)

39. Kok D. J: Crystallization and stone formation inside the nephron. *Scanning Microsc* 10, 471-484 (1996)

40. Kok D. J: Clinical implications of physicochemistry of stone formation. *Endocrinol Metab Clin North Am* 31, 855-867 (2002)

41. Jiang X. J, T. Feng, L. S. Chang, X T. Kong, G. Wang, Z. W. Zhang & Y L. Guo: Expression of osteopontin mRNA in normal and stone-forming rat kidney. *Urol Res* 26, 389-394 (1998)

42. Khan S. R, J. M. Johnson, A. B. Peck, J. G. Cornelius & P. A. Glenton: Expression of osteopontin in rat kidneys: induction during ethylene glycol induced calcium oxalate nephrolithiasis. *J Urol* 168, 1173-1181 (2002)

43. Moriyama M. T, P. A. Glenton & S. R. Khan: Expression of inter-alpha inhibitor related proteins in kidneys and urine of hyperoxaluric rats. *J Urol* 165, 1687-1692 (2001)

44. Iida S, J. Miyajima, K. Suzuki, K. Matsuoka, M. Inoue & S. Noda: Expression of heparan sulfate proteoglycan mRNA in rat kidneys during calcium oxalate nephrolithiasis. *Urol Res* 25, 361-364 (1997)

45. Asplin J. R, J. H. Parks, M. S. Chen, J. C. Lieske, F. G. Toback, S. N. Pillay, Y. Nakagawa & F. L. Coe: Reduced

crystallization inhibition by urine from men with nephrolithiasis. *Kidney Int* 56, 1505-1516 (1999)

46. Tiselius H. G, A. Hallin & B. Lindback: Crystallisation properties in stone forming and normal subjects' urine diluted using a standardised procedure to match the composition of urine in the distal part of the distal tubule and the middle part of the collecting duct. *Urol Res* 29, 75-82 (2001)

47. Jonassen J. A, L-C. Cao, T. W. Honeyman & C. R. Scheid: Mechanisms mediating oxalate-induced alterations in renal cell functions. *Crit Rev Euk Gene Exp* 13, 55-72 (2003)

48. Grases F, L. Masarova, O. Sohnel & A. Costa-Bauza: Agglomeration of calcium oxalate monohydrate in synthetic urine. *Br J Urol* 70, 240-246 (1992)

49. Kok D. J. & S. E. Papapoulos: Physicochemical considerations in the development and prevention of calcium oxalate urolithiasis. *Bone Miner Res* 20, 1-15 (1993)

50. Riese R. J, N. S. Mandel, J H. Wiessner, G. S. Mandel, C. G. Becker & J. G. Kleinman: Cell polarity and calcium oxalate crystal adherence to cultured collecting duct cells. *Am J Physiol* 262, F177-F184 (1992)

51. Mandel N: Crystal-membrane interaction in kidney stone disease. *J Am Soc Nephrol* 5, S37-S45 (1994)

52. Wiessner J. H, A. T. Hasegawa, L. Y. Hung & N. S. Mandel: Oxalate-induced exposure of phosphatidylserine on the surface of renal epithelial cells in culture. *J Am Soc Nephrol* 10, S441-S445 (1999)

53. Wiessner J. H, A. T. Hasegawa, L.Y. Hung, G. S. Mandel & N. S. Mandel: Mechanisms of calcium oxalate crystal attachment to injured renal collecting duct cells. *Kid Int* 59, 637-644 (2001)

54. Bigelow M. W, J. H. Wiessner, J. G. Kleinman & N. S. Mandel: Surface exposure of phosphatidylserine increases calcium oxalate crystal attachment to IMCD cells. *Amer J Physiol* 272, F55-62 (1997)

55. Lieske J. C. & F. G. Toback: Regulation of renal epithelial cell endocytosis of calcium oxalate monohydrate crystals. *Am J Physiol* 264, F800-F807 (1993)

56. Lieske J. C, R. Leonard, H. Swift & F. G. Toback: Adhesion of calcium oxalate monohydrate crystals to anionic sites on the surface of renal epithelial cells. *Am J Physiol* 270, F192-F199 (1996)

57. Verkoelen C. F, B. G. van der Boom, A. B. Houtsmuller, F. H. Schroder & J. C. Romijn: Increased calcium oxalate monohydrate crystal binding to injured renal tubular epithelial cells in culture. *Am J Physiol* 274, F958-F965 (1998)

58. Verhulst A, M. Asselman, V. P. Persy, M. S. Schepers, M. F. Helbert, C. F. Verkoelen, M. E. de Broe: Crystal retention capacity of cells in the human nephron: involvement of CD44 and its ligands hyaluronic acid and osteopontin in the transition

of a crystal binding into a nonadherent epithelium. *J Am Soc Nephrol* 13, 107-115 (2003)

59. Koul H, L. Kennington, G. Nair, T. Honeyman, M. Menon & C. Scheid: Oxalate-induced initiation of DNA synthesis in LLC-PK1 cells, a line of renal epithelial cells. *Biochem Biophys Res Commun* 205, 1632-1637 (1994)

60. Scheid C. R, H. K. Koul, L. Kennington, W. A. Hill, J. Luber-Narod, J. Jonassen, T. Honeyman & M. Menon: Oxalate-induced damage to renal tubular cells: a review. *Scanning Microsc* 9, 1097-1107 (1995)

61. Scheid C. R, H. Koul, W. A. Hill, J. Luber-Narod, J. Jonassen, T. Honeyman, L. Kennington, R. Kohli, J. Hodapp, P. Ayvazian & M. Menon: Oxalate toxicity in LLC-PK1 cells, a line of renal epithelial cells. *J Urol* 155, 1112-1116 (1996)

62. Scheid C, H. Koul, W. A. Hill, J. Luber-Narod, L. Kennington, T. Honeyman, J. Jonassen & M. Menon: Oxalate toxicity in LLC-PK1 cells: role of free radicals. *Kid Int* 49, 413-419 (1996)

63. Hackett R. L, P. N. Shevock & S. R. Khan: Madin-Darby canine kidney cells are injured by exposure to oxalate and to calcium oxalate crystals. *Urol Res* 22, 197-204 (1994)

64. Thamilselvan S & S. R. Khan: Oxalate and calcium oxalate crystals are injurious to renal epithelial cells: results of in vivo and in vitro studies. *J Nephrol* 11, 66-69 (1998)

65. Cao L-C, J. Jonassen, T. W. Honeyman & C. Scheid: Oxalate-induced redistribution of phosphatidylserine in renal epithelial cells: implications for kidney stone disease. *Am J Nephrol* 21, 69-71 (2001)

66. Finlayson B. & F. Reid: The expectation of free and fixed particles in urinary stone disease. *Invest Urol* 15, 442-448 (1978)

67. Kok D. J. & S. R. Khan: Calcium oxalate nephrolithiasis, a free or fixed particle disease. *Kidney Int* 46, 847-854 (1994)

68. Verkleij A. J. & J. A. Post: Membrane phospholipid asymmetry and signal transduction. *J Membr Biol* 178, 1-10 (2000)

69. Sambrano G. R. & D. Steinberg: Recognition of oxidatively damaged and apoptotic cells by an oxidized low density lipoprotein receptor on mouse peritoneal macrophages: role of membrane phosphatidylserine. *Proc Natl Acad Sci USA* 92, 1396-1400 (1995)

70. Nancollas G. H, A. Tsortos & A. Zieba: The nucleation and growth of calcium phosphate crystals at protein and phosphatidylserine liposome surfaces. *Scanning Microsc* 10, 499-507 (1996)

71. Verkoelen C. F, B. G. van der Boom, D. J. Kok & J. C. Romijn: Sialic acid and crystal binding. *Kid Int* 57, 1072-1082 (2000)

72. Lieske J. C, F. G. Toback & S. Deganello: Sialic acid-containing glycoproteins on renal cells determine nucleation of calcium oxalate dihydrate crystals. *Kidney Int* 60, 1784-1791 (2001)

73. Goswami A, P. C. Singhal, J. D. Wagner, M. Urivetzky, E. Valderrama & A D. Smith: Matrix modulates uptake of calcium oxalate crystals and cell growth of renal epithelial cells. *J Urol* 153, 206-211 (1995)

74. Sorokina E. A & J. G. Kleinman: 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, 27491-27496 (1999)

75. Khan S. R: Calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. *Urol Res* 23, 71-79 (1995)

76. Lieske J. C, M. M. Walsh-Reitz & F. G. Toback: Calcium oxalate monohydrate crystals are endocytosed by renal epithelial cells and induce proliferation. *Am J Physiol* 262, F622-630 (1992)

77. Kohjimoto Y, S. Ebisuno, M. Tamura & T. Ohkawa: Interactions between calcium oxalate monohydrate crystals and Madin-Darby canine kidney cells: endocytosis and cell proliferation. *Urol Res* 24, 193-199 (1996)

78. Campos A. H. & N. Schor: Mechanisms involved in calcium oxalate endocytosis by Madin-Darby canine kidney cells. *Braz J Med Biol Res* 33, 111-118 (2000)

79. Cheung H. S, M. T. Story & D. J. McCarty: Mitogenic effects of hydroxyapatite and calcium pyrophosphate dihydrate crystals on cultured mammalian cells. *Arthritis Rheum* 27, 668-674 (1984)

80. Cheung H. S. & D. J. McCarty: Mitogenesis induced by calcium containing crystals. Role of intracellular dissolution. *Exp Cell Res* 157, 63-70 (1985)

81. Hammes M. S, J. C. Lieske, S. Pawar, B. H. Spargo & F. G. Toback: Calcium oxalate monohydrate crystals stimulate gene expression in renal epithelial cells. *Kid Int* 48, 501-509 (1995)

82. Koul H, L. Kennington, T. Honeyman, J. Jonassen, M. Menon & C. Scheid: Activation of *c-myc* gene mediates the mitogenic effects of oxalate in LLC-PK1 cells, a line of renal epithelial cells. *Kid Int* 50, 1525-1530 (1996)

83. Jonassen J. A, R. Cooney, L. Kennington, K. Gravel, T. Honeyman & C. R. Scheid: Oxalate-induced changes in the viability and growth of human renal epithelial cells. *J Am Soc Nephrol* 10, S446-S451 (1999)

84. Dennis E. A: The growing phospholipase A2 superfamily of signal transduction enzymes. *Trends Biochem Sci* 22, 1-2 (1997)

85. Schnellman R. G, X. Yang & J. B. Carrick: Arachidonic acid release in renal proximal tubule cell injuries and death. *J Biochem Toxicol* 9, 211-217 (1994)

86. Edelstein C. L, H. Ling & R. W. Schrier: The nature of renal cell injury. *Kidney Int* 51, 1341-1351 (1997)

87. Shankland S. J: New insights into the pathogenesis of membranous nephropathy. *Kidney Int* 57, 1204-1205 (2000)

88. Baggio B. & G. Gambaro: Abnormal arachidonic acid content of membrane phospholipids -- the unifying hypothesis for the genesis of hypercalciuria and hyperoxaluria in idiopathic calcium nephrolithiasis. *Nephrol Dial Transplant* 14, 553-555 (1999)

89. Baggio B, A. Budakovic, M. A. Nassuato, G. Vezzoli, E. Manzato, G. Luisetto & M. Zaninotto: Plasma phospholipid arachidonic acid content and calcium metabolism in idiopathic calcium nephrolithiasis. *Kidney Int* 58, 1278-1284 (2000)

90. Kohjimoto Y, L. Kennington, C. R. Scheid & T. W. Honeyman: Role of phospholipase A2 in the cytotoxic effects of oxalate in cultured renal epithelial cells. *Kid Int* 56, 1432-1441 (1999)

91. Kohjimoto Y, T. W. Honeyman, J. Jonassen, K. Gravel, L. Kennington & C. R. Scheid: Phospholipase A_2 mediates immediate early genes in cultured renal epithelial cells: possible role of lysophospholipid. *Kid Int* 58, 638-646 (2000)

92. Dixon R. J & N. J. Brunskill: Lysophosphatidic acid-induced proliferation in opossum kidney proximal tubular cells: role of PI3-kinase and ERK. *Kidney Int* 56, 2064-2075 (1999)

93. Chen Y, S. Morimoto, S. Kitano, E. Koh, K. Fukuo, B. Jiang, S. Chen, O. Yasuda, A. Hirotani & T. Ogihara: Lysophosphatidylcholine causes Ca^{2+} influx, enhanced DNA synthesis and cytotoxicity in cultured vascular smooth muscle cells. *Atherosclerosis* 112, 69-76 (1995)

94. Levine J. S, J. S. Koh, V. Triaca & W. Lieberthal: Lysophosphatidic acid: a novel growth and survival factor for renal proximal tubular cells. *Am J Physiol* 273, F575-F585 (1997)

95. Kohri K, S. Nomura, Y. Kitamura, T. Nagata, K. Yoshioka, M. Iguchi, T. Yamate, T. Umekawa, Y. Suzuki, H. Sinohara & T. Kurita: Structure and expression of the mRNA encoding urinary stone protein (osteopontin). *J Biol Chem* 268, 15180-15184 (1993)

96. Umekawa T, K. Kohri, T. Kurita, S. Hirota, S. Nomura & Y. Kitamura: Expression of osteopontin messenger RNA in the rat kidney on experimental model of renal stone disease. *Biochem Molec Biol Int* 35, 223-230 (1995)

97. Lieske J. C, M S. Hammes, J. R. Hoyer & F. G. Toback: Renal cell osteopontin production is stimulated by calcium oxalate monohydrate crystals. *Kidney Int* 51, 679-686 (1997)

98. Yasui T, K. Fujita, S. Sasaki, M. Sato, M. Sugimoto, S. Hirota, Y. Kitamura, S. Nomura & K. Kohri: Expression of bone matrix proteins in urolithiasis model rats. *Urol Res* 27, 255-261 (1999)

99. Marengo S. R, D. H. Chen, H. L. Kaung, M. I. Resnick & L. Yang: Decreased renal expression of the putative calcium oxalate inhibitor Tamm-Horsfall protein in the ethylene glycol rat model of calcium oxalate urolithiasis. *J Urol* 167, 2192-2197 (2002)

100. Katsuma S, S. Shiojima, A. Hirasawa, K. Takagaki, Y. Kaminishi, M. Koba, Y. Hagidai, M. Murai, T. Ohgi, J. Yano & G. Tsujimoto: Global analysis of differentially expressed genes during progression of calcium oxalate nephrolithiasis. *Biochem Biophys Res Commun* 296, 544-552 (2002)

101. Scheid C, T. Honeyman, Y. Kohjimoto, L-C. Cao & J. Jonassen: Oxalate-induced changes in renal epithelial cell function: role in stone disease. *Molec Urol* 4, 371-382 (2000)

102. Iida S, A. B. Peck, K. J. Byer & S. R. Khan: Expression of bikunin mRNA in renal epithelial cells after oxalate exposure. *J Urol* 162, 1480-1486 (1999)

103. Kume N, M. I. Cybulsky & M. A. Gimbrone Jr: Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J Clin Invest* 90, 1138-1144 (1992)

104. Kume N. & M. A. Gimbrone Jr: Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. *J Clin Invest* 93, 907-911 (1994)

105. Zhu Y, JH-C Lin, H-L. Liao, L. Verna & M. B. Stemerman: Activation of ICAM-1 promoter by lysophosphatidylcholine: possible involvement of protein tyrosine kinases. *Biochim Biophys Acta* 1345, 93-98 (1997)

106. Nishi E, N. Kume, H. Ochi, H. Moriwaki, Y. Wakatsuki, S. Higashiyama, N. Taniguchi & T. Kita: Lysophosphatidylcholine increases expression of heparin-binding epidermal growth factor-like growth factor in human T lymphocytes. *Circ Res* 80, 638-644 (1997)

107. Rong J. X, J. W. Berman, M. B. Taubman & E. A. Fisher: Lysophosphatidylcholine stimulates monocyte chemoattractant protein-1 gene expression in rat aortic smooth muscle cells. *Arterioscler Thromb Vasc Biol* 22, 1617-1623 (2002)

108. Umekawa T, N. Chegini & S. R. Khan: Oxalate ions and calcium oxalate crystals stimulate MCP-1 expression by renal epithelial cells. *Kidney Int* 61, 105-112 (2002)

109. Viedt C. & S. R. Orth: Monocyte chemoattractant protein-1(MCP-1) in the kidney: does it more than simply attract monocytes? *Nephrol Dial Transplant* 17, 2043-2047 (2002)

110. de Water R, C. Noordermeer, A. B. Houtsmuller, A. L. Nigg, T. Stijnen, F. H. Schroder & D. J. Kok: Role of macrophages in nephrolithiasis in rats: an analysis of the renal interstitium. *Am J Kidney Dis* 36, 615-625 (2000)

111. Fang X, S. Gibson, M. Flowers, T. Frui, R. C. Bast Jr & G. B. Mills: Lysophosphatidycholine stimulate activator protein 1 and the c-Jun N-terminal kinase activity. *J Biol Chem* 272, 13683-13689 (1997)

112. Ueno Y, N. Kume, S. Miyamoto, M. Morimoto, H. Kataoka, H. Ochi, E. Nishi, H. Moriwaki, M. Minami, N. Hashimoto & T. Kita: Lysophosphatidylcholine phosphorylates CREB and activates the jun2TRE site of c-jun promoter in vascular endothelial cells. *FEBS Lett* 457, 241-245 (1999)

113. Yamakawa T, S. Eguchi, Y. Yamakawa, E. D. Motley, K. Numaguchi, H. Utsunomiya & T. Inagami: Lysophosphatidylcholine stimulates MAP kinase activity in rat vascular smooth muscle cells. *Hypertens* 31, 248-253 (1998)

114. Cieslik K, A. Zembowicz, J.-L. Tang & K. K. Wu: Transcriptional regulation of endothelial nitric-oxide synthase by lysophosphatidylcholine. *J Biol Chem* 273, 14885-14890 (1998)

115. Morimoto M, N. Kume, S. Miyamoto, Y. Ueno, H. Kataoka, M. Minami, K. Hayashida, N. Hashimoto & T. Kita: Lysophosphatidylcholine induces early growth response factor-1 expression and activates the core promoter of PDGF-A chain in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 21, 771-776 (2001)

116. Masamune A, Y. Sakai, M. Yoshida, A. Satoh, K. Satoh & T. Shimosegawa: Lysophosphatidylcholine activates transcription factor NF-kappaB and AP-1 in AR42J cells. *Dig Dis Sci* 46, 1871-1881 (2001)

117. Miller C, L. Kennington, R. Cooney, T. Honeyman, Y. Kohjimoto, L-C. Cao, J. Pullman, J. Jonassen & C R. Scheid: Oxalate toxicity in renal epithelial cells: characteristics of apoptosis and necrosis. *Toxicol Appl Pharm* 162, 132-141 (2000)

118. Cao L-C, T. Honeyman, J. Jonassen & C. Scheid: Oxalate-induced ceramide accumulation in Madin-Darby canine kidney and LLC-PK1 cells. *Kidney Int* 57, 2403-2411 (2000)

119. Kolesnick R. N & M. Kronke: Regulation of ceramide production and apoptosis. *Annu Rev Physiol* 60, 643-665 (1998)

120. Jayadev S, C. M. Linardic & Y. A. Hannun: Identification of arachidonic acid as a mediator of sphingomyelin hydrolysis in response to tumor necrosis factor alpha. *J Biol Chem* 269, 5757-5763 (1994)

121. Riboni L, P. Viani, R. Bassi, A. Prinetti & G. Tettamanti: The role of sphingolipids in the process of signal transduction. *Prog Lipid Res* 36, 153-195 (1997)

122. Hashizume T, K. Kitatani, T. Kageura, M. Hayama, S. Akiba & T. Sato: Ceramide enhances susceptibility of membrane phospholipids to phospholipase A2 through

modification of lipid organization in platelet membranes. *Biol Pharm Bull* 22, 1275-1278 (1999)

123. Hannun Y. A: The sphingomyelin cycle and the second messenger function of ceramide. *J Biol Chem* 269, 3125-3128 (1994)

124. Khan S. R, K. J. Byer, S. Thamilselvan, R. L. Hackett, W. T. McCormack, N. A. Benson, K. L. Vaughn & G. W. Erdos: Crystal-cell interaction and apoptosis in oxalate-associated injury of renal epithelial cells. *J Am Soc Nephrol* 10, S457-S463 (1999)

125. Sarica K, F. Yagci, K. Bakir, A. Erbagci, S. Erturhan & R. Ucak: Renal tubular injury induced by hyperoxaluria: evaluation of apoptotic changes. *Urol Res* 29, 34-37 (2001)

126. Crompton M: The mitochondrial permeability transition pore and its role in cell death. *Biochem J* 341, 233-249 (1999)

127. Kurien T. B. & R. Selvam: Induction of lipid peroxidation in calcium oxalate stone formation. *Indian J Exp Biol* 27, 450-453 (1989)

128. Muthukumar A. & R. Selvam: Renal injury mediated calcium oxalate nephrolithiasis: role of lipid peroxidation. *Renal Fail* 19, 401-408 (1997)

129. Thamilselvan S, R. L. Hackett & S. R. Khan: Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. *J Urol* 157, 1059-1063 (1997)

130. Selvam R: Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. *Urol Res* 30, 35-47 (2002)

131. Muthukumar A. & R. Selvam: Role of glutathione on renal mitochondrial status in hyperoxaluria. *Mol Cell Biochem* 185, 77-84 (1998)

132. Huang H. S, M. C. Ma, J. Chen & C. F. Chen: Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. *J Urol* 167, 2584-2593 (2002)

133. Khand F. D, M. P. Gordge, W. G. Robertson, A. A. Noronha-Dutra & J. S. Hothersall: Mitochondrial superoxide production during oxalate-mediated oxidative stress in renal epithelial cells. *Free Radic Biol Med* 32, 1339-1350 (2002)

134. Thamilselvan S, K. J. Byer, R. L. Hackett & S. R. Khan: Free radical scavengers, catalase and superoxide dismutase provide protection from oxalate-associated injury to LLC-PK1 and MDCK cells. *J Urol* 164, 224-229. (2000)

135. Thamilselvan S, S. R. Khan & M. Menon: Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. *Urol Res* 31, 3-9 (2002)

136. Hsieh N, C. H. Shih, H. Y. Chen, M. C. Wu, W. C. Chen & C. W. Li: Effects of Tamm-Horsfall protein on the protection of MCDK cells from oxalate induced free radical injury. *Urol Res* 31, 10-16 (2003)

137. Ruvolo P. P, X. Deng, B. K. Carr & W. S. May: A functional role for mitochondrial protein kinase Calpha in Bcl2 phosphorylation and suppression of apoptosis. *J Biol Chem* 273, 25436-25442 (1998)

138. Cao L-C, T. W. Honeyman & C. R. Scheid. Role of mitochondria in oxalate actions on renal cells. In: Eurolithiasis. 9th European Symposium of Urolithiasis. pp. 110-111. Eds: Kok D. J. , Romijn H. C. , Verhagen C. M. S., Verkoelen C. F., Shaker Publishing, Maastricht, the Netherlands. (2001)

139. Strzelecki T, B. R. McGraw, C. R. Scheid & M. Menon: Effect of oxalate on function of kidney mitochondria. *J Urol* 141, 423-427 (1989)

140. Loeffler M. & G. Kroemer: The mitochondrion in cell death control: certainties and incognita. *Exp Cell Res* 256, 19-26 (2000)

141. Petronelli V, D. Penzo, L. Scorrano, P. Bernardi & F. Di Lisa: The mitochondrial permeability transition, release of cytochrome c and cell death. Correlation with the duration of pore openings in situ. *J Biol Chem* 276, 12030-12034 (2001)

142. Khan S. R, F. Atmani, P. Glenton, Z. Hou, D. R. Talham & M. Khurshid: Lipids and membranes in the organic matrix of urinary calcific crystals and stones. *Calcif Tissue Int* 59, 357-365 (1996)

143. Evan A. P, J. E. Lingeman, F. L. Coe, J. H. Parks, S. B. Bledsoe, Y. Shao, A. J. Sommer, R. F. Paterson, R. L. Kuo & M. Grynpas: Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. *J Clin Invest* 111, 607-616 (2003)

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