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,
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Table 1. Macromolecules Affecting Crystal Formation

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

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 of crystals (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 vitro likely differ from those in vivo 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,
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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 renal cell injury, produced by exposure to elevated oxalate levels (59-64), also leads to increase in crystal attachment of crystals (53, 65) which can subsequently be internalized and deposited in interstitial spaces and/or remain at the cell surfaces serving as nidus 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 surfaces 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...
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<table>
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<th>Gene</th>
<th>Model system</th>
<th>Function/ potential role in stone disease</th>
<th>References</th>
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<td>rat stone models, renal cell culture</td>
<td>stone matrix, inhibitor of crystallization</td>
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<td>renal cell cultures</td>
<td>renal injury marker</td>
<td>101</td>
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<td>rich in cysteine-SPARC)</td>
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<td>Inhibitor of crystallization</td>
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<td>ECM accumulation/ remodeling</td>
<td>81</td>
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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 e-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
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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 bel-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 thiol 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

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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.

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Abbreviations: MDCK: Madin Darby Canine Kidney; Lyso-PC: Lysophosphatidylcholine; ROS: Reactive oxygen species; PLA2: Phospholipase A2.

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