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The Role of Urinary Kidney Stone Inhibitors and Promoters in the Pathogenesis of Calcium Containing Renal Stones

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Abstract

Urinary stone disease is an ailment afflicting human kind for many centuries. It can affect up to a quarter the population in certain geographic areas and hence poses a significant health problem. Various aetiological factors have been attributed to stone formation – hereditary, dietary, geographical, infective etc. Approximately 85% of the stones in human are calcium stones comprising oxalate and phosphate, either alone or combined. Though supersaturation of stone forming salts in urine is essential, abundance of these salts by itself will not always result in stone formation.

The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes – nucleation, crystal growth, crystal aggregation and crystal retention. Various substances in the body have an effect on one or more of the above stone forming processes, thereby influencing a person's ability to promote or prevent stone formation. Promoters of stone formation facilitate stone formation whilst inhibitors prevent it. Low urine volume, low urine pH, calcium, sodium, oxalate, and urate are known to promote stone formation. Many inorganic (eg. Citrate, magnesium) and organic (eg. Urinary prothrombin fragment 1, glycosaminoglycans, osteopontin) substances are known to inhibit stone formation. Organic inhibitory compounds adsorb to the surface of the crystal, thereby inhibiting crystal growth and nucleation. This review presents a comprehensive account of the basic principles of stone formation and role of urinary inhibitors/promoters in calcium oxalate crystallisation.

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1. Introduction

Urolithiasis is a global problem afflicting human beings for several centuries. The annual incidence of urolithiasis in the western world is 0.5% [1]. The life time risk of developing urolithiasis is about 10–15% in the western world, but can be as high as 20–25% in the middle east [1]. The prevalence of urolithiasis in the United States is increasing [2]. The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 years, and 50% at 10 years [3]. Since the first description of Randall's plaques in the late 1930s, several authors have attempted to explore and advance the process of stone formation in humans [4]. Kidney stone disease is a common chronic disorder in humans and the most common type of renal stone is made of calcium oxalate (CaOx). The present review aims at summarising important aspect of CaOx stone formation and briefly discusses factors that inhibit or promote the formation of crystals in urine.

CaOx stones are made of CaOx monohydrate (COM) and CaOx dihydrate (COD) crystals. COM, the thermodynamically most stable form, is observed more frequently in clinical stones than COD, at a ratio of >2:1. As a consequence of the essential homeostatic conservation of water, normal urine is supersaturated with respect to crystalline components, and this empirically suggests the existence of physiological mechanisms that actively inhibit urinary crystallization of calcium salts. Various inhibitory macromolecules e.g. crystal matrix protein, bikunin, Tamm-Horsfall protein (THP), and urinary osteopontin (OPN) have now been isolated and identified from both normal urine and kidney stones. In addition, anatomic factors can also contribute to the development of stone disease.

2. Pathophysiology

Calcium stone formation involves different phases of increasing accumulation of CaOx and calcium phosphate (CaP) – nucleation, crystal growth, crystal aggregation and crystal retention.

2.1. Nucleation

Nucleation is the formation of a solid crystal phase in a solution. It is an essential step in renal stone formation [5–7]. The term **supersaturation** refers to a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances. The level of supersaturation of a salt is expressed as the ratio between the actual ion-activity product (AP_{salt}) and the solubility product (SP_{salt}). The ion-activity of a salt is calculated from the free ion concentrations and the activity coefficients corresponding to the charge of the ions in the salt. The point at which saturation of a solution is reached, and crystallization begins is commonly known as thermodynamic solubility product (Ksp). Urine contains inhibitors of crystallisation and can hold large concentrations of solute above the Ksp, a metastable state. If the concentration of solute increases further and a point is reached where it cannot be held in solution, this concentration is known as K_F, which is the point of formation of product in urine. The process of nucleation in a pure solution is known as homogeneous nucleation [5]. In secondary nucleation, new crystals deposit on pre-existing crystal surfaces of similar type. Secondary nucleation results in the 'mass production' of crystals. Epitaxy is a process where by material of one crystal type is precipitated upon the surface of another whose lattice dimensions are almost identical [8]. Epitaxy is clinically important in the formation calcium oxalate stones; the presence of uric acid crystals may promote formation of calcium oxalate stones. These 2 processes are closely related to heterogeneous nucleation. Urine is not a pure solution and nucleation in urine often occurs over an existing surface, or an alternative structure. This process is called heterogeneous nucleation. Heterogeneous nucleation sites in urine can be epithelial cells, red blood cells, cell debris, urinary casts, other crystals and bacteria (Fig. 1). Hyperuricosuria can promote calcium oxalate crystallisation without epitaxy (so-called salting-out effect). The formation product of heterogeneous nucleation is the ion-activity product at which facilitated crystal formation occurs and the formation product of homogeneous nucleation is the ion-activity product needed for spontaneous formation of crystals.

Although crystallogenesis is essential to stone formation, CaOx crystal growth rates are sluggish, to the extent that during typical urine transit times single crystals will not grow large enough to become lodged in the terminal collecting duct of the kidney [9]. Crystallisation represents the first phase of urinary stone formation. Stones results from a phase change in which dissolved salts condense into solids and this transformation is influenced by supersaturation (SS). If SS is <1, crystals of substance will dissolve but crystals can form and grow if SS >1 and urine SS >1 is metastable and excess dissolved substance will precipitate. Biomineralisation requires an organic material and organic matrix accounts for approximately 2.5% of the weight of the
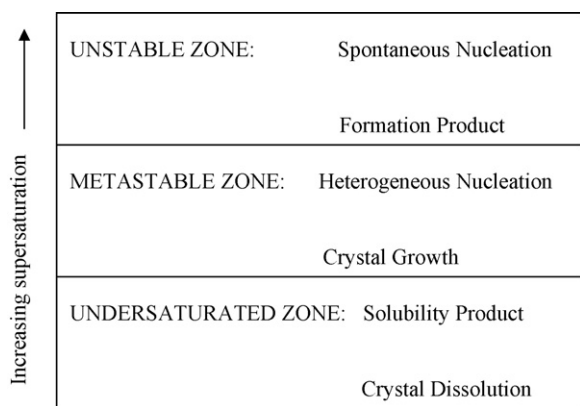
Fig. 1 – Relative supersaturation and calcium crystal nucleation.

stone [10]. Crystal aggregation and attachment of crystals or aggregates to an alternative nidus such as renal epithelial cells are critical processes in stone formation. Standard laws of physical chemistry can explain the occurrence of crystals in a static solution but urine in the kidney is not static. Different theories are proposed to explain the process of nucleation in the kidney (Fig. 2). Finlayson and Ried calculated that free particle stone formation was mathematically impossible and proposed that stone disease required the adherence of crystals to the renal epithelium (fixed particle nucleation) but Kok and Khan using modern computational methods have proposed that aggregation of free crystals can result in urinary microliths large enough to occlude collecting ducts (free particle nucleation) [11].

Where is the site of initial crystal deposition? There has been increasing interest to find an answer to this question since the early suggestions of

Randall's plaque. In a landmark study, Evan et al [12,13] performed kidney biopsies from area adjacent to Randall's plaque on stone forming patients. In hypercalciuric calcium oxalate stone formers, they observed initial calcium phosphate deposition in the basement membrane of the thin limbs of the loop of Henle. They also found further extension to the vasa recta, then to the interstitial tissue surrounding the ducts of Bellini, and finally proceeding to the urothelium of the papillary tip. In patients with hyperoxaluria resulting from intestinal bypass initial crystal deposition was found in the lumens of a few collecting ducts and crystals were hydroxyapatite. No crystal deposits were seen in the interstitum or around the thin loop of Henle. These findings raised further questions; why does the initial crystallisation occur in these sites and why initial crystals are composed of calcium phosphate? Bushinsky [14] hypothesied that physiological changes within the interstitium and vasa recta following ingestion and absorption of dietary calcium, may decrease bicarbonate removal from the medullary interstitium. The resultant increased pH would reduce the solubility of calcium phosphate complexes and probably an extracellular matrix protein may promote heterogeneous nucleation. Stoller et al [15] suggested a possible intravascular phenomenon in the vasa recta may affect the adjacent urinary collecting system and may promote initial solid phase.

2.2. Crystal growth

After nucleation, crystal growth is the next major step of stone formation. What causes crystals to "grow"? The driving force for crystallization is a
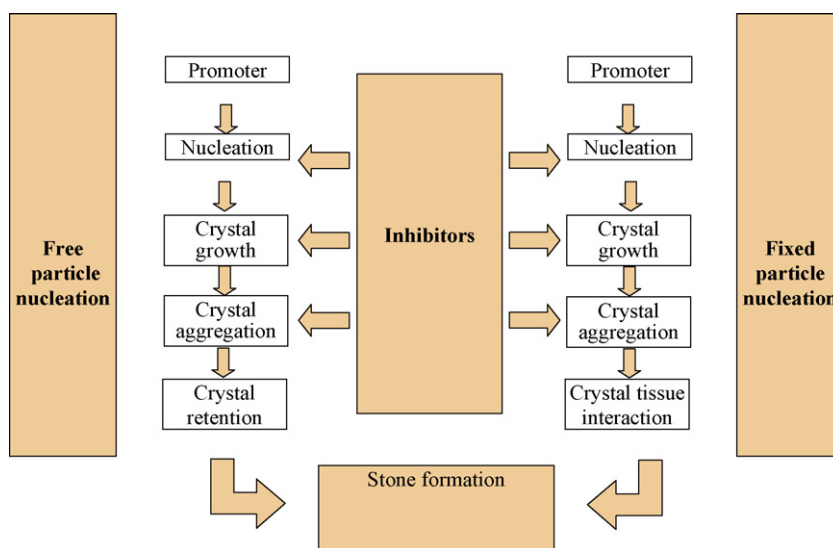

Fig. 2 – Summary of the possible steps in calcium stone formation.

reduction in the potential energy of the atoms or molecules when they form bonds to each other. The crystal growth process starts with the nucleation stage. Several atoms or molecules in a super-saturated liquid start forming clusters; the bulk free energy of the cluster is less than that of the liquid. The total free energy of the cluster is increased by the surface energy (surface tension), however, this is significant only when the cluster is small. Crystal growth is determined by the molecular size and shape of the molecule, the physical properties of the material, SS levels, pH, and defects that may form in the crystal's structure. Crystal growth is one of the prerequisites for particle formation. Using the powerful atomic-force microscope (AFM), Laboratory researchers are discovering complex growth mechanisms and three-dimensional structures of solution-based crystals [16].

2.3. Crystal aggregation (crystal agglomeration)

In this process crystals in solution stick together and form a larger particle. Aggregation of particles in solution is determined by a balance of forces, some with aggregating effects and some with disaggregating effects. A small interparticle distance increases attractive force and favours particle aggregation. In addition, Tamm-Horsfall glycoprotein and other molecule may act as glue and increase viscous binding [17]. Furthermore, aggregate may be stabilised by solid bridges formed by crystalline material connecting two particles. The main force that inhibits aggregation is the repulsive electrostatic surface charge, known as Zeta potential. In various steps of stone formation, crystal aggregation is a more important factor than nucleation and growth because aggregation occurs within seconds [18].

It is a widely held belief that the process of calcium stone formation starts as a precipitation of CaP in the loop of Henle or the distal part of the distal tubule [6,19]. In normal physiochemical condition repulsion occurs between the CaP crystals and tubular cells and may result in elimination of small CaP crystal by dissolution or spontaneous passage in urine. Primary nucleation of CaOx crystal is induced by CaP, small crystals are then excreted in the urine. In addition, internalisation and macrophage destruction of CaOx crystals occurs in the interstitial tissue. Pathological crystallisation results from the effects of secreted macromolecules secreted in the urine and secreted by the brush border of proximal tubular cells on the interaction between the tubular cells and crystals [20]. Experimental studies have demonstrated that injury from free radicals may

result in sloughed membrane fragments in the tubular lumen and this may providing a suitable surface for nucleation of CaP and oxalate [7]. This event may cause formation of masses of crystals by growth and aggregation followed by adherence of CaP crystals aggregates to the tubular surface. Newly formed crystals adhere to the tubular cell surface and the cellular responses that follow could result in crystal retention and thereby set in motion a series of events that lead to pathologic renal calcification. Dissolution of CaP in acid urine causes a high level of SS with CaOx [19] and nucleation of CaOx facilitates the formation of a large mass of CaOx and phosphate attached to the tubular cell wall.

2.4. Crystal retention

Urolithiasis requires formation of crystals followed by their retention and accumulation in the kidney. Crystal retention can be caused by the association of crystals with the epithelial cells lining the renal tubules. Crystal formation predominantly depends on the composition of the tubular fluid; crystal retention might depend on the composition of the renal tubular epithelial cell surface [21,22]. A non-adherent surface of the distal tubules, collecting ducts, ureters, bladder, and the urethra may provide a natural defence mechanism against crystal retention, and may become defective when the anti-adherence properties are compromised. In a cell culture model, Verhulst et al observed upregulated cell surface expression of hyaluronic acid, osteopontin, and their receptor CD44, as well as the formation of a hyaluronic acid-dependent cell coat, and suggested that it may play a crucial role in the process of crystal retention [23].

3. Stone inhibitors and promoters

Inhibitors are defined as molecules that increase the SS required to initiate nucleation, decrease crystal growth rate and aggregation, and inhibit secondary nucleation. In contrast promoters reduce the formation product of the supersaturated solution. An imbalance between urinary-promoting and -inhibiting factors has been suggested as more important in urinary stone formation than a disturbance of any single substance. These substances include inorganic compounds, proteins, and glycosamino-glycans (Table 1). Abnormal function and or concentration of these compounds in the urine may modify physiochemical conditions to promote stone formation [24,25].

Table 1 – Stone promoting and inhibiting factors

Promoting factors	Inhibiting factors
Calcium	**Inorganic**
Sodium	Citrate
Oxalate	Magnesium
Urate	Pyrophosphate
Cystine	**Organic**
Low urine pH	Tamm-Horsfall protein
Tamm-Horsfall protein	Urinary Prothrombin fragment 1
Low urine flow	Protease inhibitor: inter α inhibitor
	Glycosaminoglycans
	Osteopontin (Uropontin)
	Renal lithostathine
	Other Bikunin, Calgranulin
	High urine flow

3.1. Inhibitors

Inhibitors of calcium stone formation prevent crystal growth and aggregation by coating the surface of growing calcium crystals or by complexing with calcium and oxalate (Table 2).

3.1.1. Citrate

Citric acid is a tricarboxylic acid that circulates in blood complexed to calcium, magnesium and sodium at physiological pH of 7.4. Most of the circulating citrate is derived from endogenous oxidative metabolism. It is filtered freely through the glomelurus [26]. Approximately 75% of the filtered citrate is reabsorbed in the proximal convoluted tubule. Apart from idiopathic causes, other aetiological factors of hypocitraturia are – use of drugs like acetazolamide and thiazides, renal tubular acidosis, urinary tract infection, hypokalemia, hypomagnesemia and inflammatory bowel disease. Thiazide diuretics may induce hypocitraturia owing to hypokalemia with resultant intracellular acidosis. Hypocitraturia is a common disorder occurring in >50% of patients with nephrolithiasis [27]. Citrate has been widely studied for its stone inhibiting action in urine and it has been found to be particularly effective against the calcium oxalate

and phosphate stones [28,29]. Citrate appears to alter both calcium oxalate monohydrate and calcium phosphate crystallisation. The most established effect of citrate in urine is to complex with calcium thereby reducing the concentration of CaOx. This appears to be due to effects directly on the crystal surface rather than to an alteration of the availability of free calcium. Citrate is an inhibitor of calcium crystallisation and has been shown to be an important inhibitor of CaOx agglomeration [30]. Citrate also increases the CaOx aggregation inhibitory activity of other urine macromolecules (eg, THP) and may reduce the expression of urinary (osteopontin) OPN, which is an important component of the protein matrix of urinary stones. In addition, urinary citrate excretion can increase urinary pH, which is a factor in the calcium-citrate-phosphate complex formation. Finally, urinary alkalisation by potassium citrate increases the solubility of uric acid and thus prevents the salting out of calcium oxalate by urate.

The most important determinant of renal tubular reabsorption of citrate is acid-base balance. Systemic acidosis increases citrate reabsorption from the renal tubules because of an increased demand of the body (resulting in a lower urinary citrate excretion), and conversely, alkalosis or alkali-loading from the GI tract decreases citrate reabsorption (thus increasing urinary citrate excretion) [31,32]. Urinary citrate excretion is reported to be correlated to the urinary volume, calcium, magnesium excretion and GI-alkali load. Theoretically, a high intake of protein and consequent high amino acid-load may enhance acidosis tendency and promote compensatory renal tubular absorption of citrate, resulting in low urinary citrate.

More recently, He et al. reported that the Na⁺/dicarboxylate co-transporter 1 (hNaDC1), one of sodium-citrate co-transporters, was expressed highly in the kidneys of stone formers with hypocitraturia, and concluded that the upregulation of hNaDC1 mRNA induced by intake of protein-rich

Table 2 – Effects of urinary crystallisation inhibitors

	Growth inhibition	Nucleation inhibition	Aggregation inhibition	Cell adherence
Osteopontin	Inhibitor	Inhibitor	Inhibitor/promoter	Inhibitor
Glycosaminoglycans	Inhibitor	Inhibitor	Inhibitor	Inhibitor
Bikunin	Inhibitor		Inhibitor	Inhibitor
Human urinary trefoil factor (THF1)	Inhibitor			
THP	Promoter?	Promoter	Inhibitor/promoter	
Renal lithostathine	Inhibitor			
Citrate	Inhibitor			
Pyrophosphate	Inhibitor			
Magnesium	Inhibitor			

foods may be an important cause of hypocitraturia [33]. Citrate therapy has been used in randomised studies [34–36] and a reduced recurrence was observed. Guerra et al reported poor inhibitory effect of citrate and magnesium in highly concentrated urine, in contrast, urinary dilution improved inhibitory effects [37].

3.1.2. Pyrophosphates

At low concentrations, 16 μM, pyrophosphate inhibits COM crystal growth by 50%. The urinary pyrophosphate level is in the range of 20–40 μM and therefore, theoretically levels are high enough to inhibit CaOx and CaP crystallisation [38]. Pyrophosphate and diphosphate have shown to inhibit the precipitation of CaP, where as diphosphates also inhibits the growth of apatite crystals [39]. Pyrophosphate will reduce the absorption of calcium in the intestine and this action probably mediated by formation of 1.25 $(OH)_2$ – vitamin D. Sharma et al reported low 24-hour urinary excretion of pyrophosphate in stone formers (50.67 ± 2.16 μmol/24 h) as compared to normal subjects (71.46 ± 5.46 μmol/24 h) ($p < 0.01$) [40].

Oral administration of orthophosphate has shown little benefit in prevention of stone recurrence. Conversely, patients treated in a randomised, placebo-controlled study recorded increased stone formation in the orthophosphate treated group over placebo treated subjects over a 3-year period. There is a lack of scientific evidence to support preventive role of orthophosphate [41].

3.1.3. Magnesium

Magnesium is the fourth most abundant mineral in the body and is largely found in bones. Dietary magnesium is absorbed in the small intestines and excreted through the kidney. Only 1% of total body magnesium circulates in blood [42]. In a supersaturated CaOx solution 2 mmol/L magnesium reduced particle number by 50% [43]. Magnesium can form complexes with oxalate and decreases SS. Oral intake of magnesium will decrease the oxalate absorption and urinary excretion, in a manner similar to calcium by binding to oxalate in the gut [44]. Magnesium supplementation in subjects with magnesium deficiency increases the excretion of citrate in urine [45]. However, there is little evidence to recommend magnesium therapy in patients with urolithiasis.

3.1.4. Inter-alpha-trypsin inhibitor family of proteins

Inter-α-inhibitor (IαI) belongs to the Kunitz-type protein superfamily, a group of proteins possessing a common structural element (kunin) and the ability to inhibit serine proteases [46]. IαI is a glycoprotein composed of 2 heavy chains (HC1 and HC2) and one light chain, also known as bikunin [47]. A possible role of IαI in stone disease can be traced as far back as 1909, when Bauer and Reich demonstrated that the proteolytic activity of trypsin was inhibited by urine [48]. In 1990, Sørensen et al. isolated from human urine a protein that inhibited CaOx crystal growth in an inorganic crystallization system [49]. Available evidence suggest that the proteins studied by various authors were either bikunin itself, or a fragment of bikunin, a supposition supported in a recent report that uronic-acid-rich protein is indeed bikunin [50]. Bikunin circulates free in plasma and is excreted in urine where it degrades further to fragments HI14 and HI8. Bikunin, a Kunitz-type protease inhibitor found in human amniotic fluid and urine, exhibits anti-inflammatory and antimetastatic functions in animals [51] and humans [52]. It is expressed mainly in the proximal tubules and the thin descending segment near the loop of Henle. It may contribute to the regulation of crystal adhesion and retention within tubules during kidney stone formation [53]. Furthermore, the potent inhibition of CaOx crystal growth by these proteins, coupled with the known presence of bikunin and its fragments in urine, suggested the possible existence of a relationship between IαI and CaOx stone formation [54].

3.1.5. Osteopontin (Uropontin)

Osteopontin (OPN) is a negatively-charged aspartic acid rich protein that inhibits growth of CaOx crystals in a supersaturated solution. OPN is intimately involved in the regulation of both physiological and pathological mineralization. OPN is a phosphorylated protein of wide tissue distribution that is found in association with dystrophic calcification including in the organic matrix of kidney stones. OPN is synthesised within the kidney and present in the human urine at levels in excess of 100 nM. The bone derived and kidney derived forms of this protein appear to be very similar to amino acid sequence. It is involved in various biologic processes including inflammation, leukocyte recruitment, wound healing and cell survival [55,56].

In vitro studies suggest that OPN may inhibit the nucleation, growth and aggregation of CaOx crystals. In addition it also inhibits the crystal adhesion to cultured epithelial cells [57]. Wesson et al observed that it may direct CaOx crystallisation to the CaOx dehydrate phase rather than the CaOx monohydrate (COM) phase, the dehydrate being less adherent to renal tubular epithelial cells [58]. Clinical studies to

date are inconclusive regarding the relationship between OPN and renal stone disease. Some investigators have reported decreased concentrations of OPN in urine from stone formers compared to normal individuals [59], while others have not [60]. A single base mutation in the OPN gene is seen at significantly higher incidence in patients with recurrent stone formation or familial nephrolithiasis [61]. These results suggest that OPN may play an important role in protecting the kidney from stone formation, but a cause and effect relationship in humans has not been established.

3.1.6. Urinary prothrombin fragment 1

The blood clotting factor prothrombin is degraded into three fragments – thrombin, fragment 1 and fragment 2. Fragment 1 is excreted in urine and is named Urinary prothrombin fragment (UPTF1) and is a potent inhibitor of CaOx stone formation in vitro [62].

The organic matrix of CaOx crystals contains UPTF1, providing evidence that links the role of blood coagulation proteins with urolithiasis. UPTF1 is an important inhibitor of CaOx crystal aggregation and adherence of crystals to renal cells. In South Africa the incidence of urolithiasis in blacks is significantly less compared to whites. UPTF1 from the black population has a superior inhibitory activity over UPTF1 from the white population [63]. Further studies indicate that sialylated glycoforms of UPTF1 afford protection against CaOx stone formation, possibly by coating the surface of CaOx crystals, [64].

3.1.7. Tamm-Horsfall protein

Tamm and Horsfall isolated a mucoprotein from the human urine nearly 50 years ago, and showed that the protein was able to interact and inhibit viral haemagglutination [65]. Tamm-Horsfall protein (THP), also known as uromucoid, is an 80-kDa glycoprotein synthesized exclusively in the thick ascending limb of the loop of Henle's loop (TAL) with exception the of the macula densa. THP is the most abundant protein in the urine of normal mammals. THP production ranges from 30 to 60 mg/24 h in humans [66]. THP may be involved in the pathogenesis of cast nephropathy, urolithiasis, and tubulointerstitial nephritis [67]. There is good evidence that the excessive intake of animal protein predisposes to stone disease [68]. Interestingly, a significant increase in urinary THP is seen following administration of with high-protein diet in rats [66]. Mo et al by ablating the murine THP gene established that THP is on the first line of host defences against both renal stone formation and bacterial infection [69]. In

human studies, however, no affect of dietary habits (high fluid intake, dairy calcium and protein) is seen on THP levels [70]. Using Fourier-transform infrared spectroscopy, Knörle et al 1994 reported that inhibitory THP from healthy persons was highly glycosylated with sialic acid compared to promoting THP from recurrent stone formers [71]. THP function and structure may be influenced by other factors such as THP from diabetic patients for example appears to aggregate more easily, possibly due to altered glycosylation [72].

Much controversy exists about whether THP is a promoter or an inhibitor of crystal aggregation. Most authors believe that it is an effective inhibitor of COM crystal aggregation in solutions with high pH, low ionic strength and low concentration of divalent ions and THP. In contrast, with low pH, high concentrations of calcium, sodium, and hydrogen ions as well as low THP, inhibitory activity is lost and it may even become a promoter of aggregation [17]. Self-aggregation of THP may promote either heterogenous nucleation or formation of a protein and crystalline mass large enough to block the tubular lumen.

3.1.8. Glycosaminnoglycans

Glycosaminnoglycans (GAGs) have been identified as one of the macromolecules present in the stone matrix. chondroitin sulphate, heparin sulphate and hyaluronic acid are excreted in the urine [73]. Recently, the main GAGs found in stone matrix were identified as heparan sulphate and hyaluronic acid. They are thought to play an important role in CaOx crystallization [74]. GAGs concentration in the urine is too low to decrease calcium SS. In vitro, GAGs have shown to act as inhibitors of CaOx crystal growth and crystal aggregation. However, investigators have failed demonstrate any qualitative and/or quantitative significant difference in total excretion of GAGs between stone formers and controls [75].

3.1.9. Renal lithostathine

Lithostathine is a protein of pancreatic secretion inhibiting calcium carbonate crystal growth. A protein immunologically related to lithostathine is actually present in urine of healthy subjects and in renal stones, renal lithostathine (RL) [76]. Immunocytochemistry of kidney sections localized the protein to cells of the proximal tubules and thick ascending limbs of the loop of Henle. Because of its structural and functional similarities with pancreatic lithostathine, it was called renal lithostathine. RL seems to control growth of calcium carbonate crystals. Several reports showing the presence of

calcium carbonate (CaCO3) in renal stones suggested that crystals of CaCO3 might be present in the early steps of stone formation. Such crystals might therefore promote CaOx crystallization from supersaturated urine by providing an appropriate substrate for heterogeneous nucleation [77,78].

3.1.10. Other macromolecules

Human urinary trefoil factor (THF1) belongs to the trefoil factor family proteins. It is synthesised by mucosal epithelial cells and is expressed in gastric mucosa. It has been described as an antiapoptotic agent with mitogenic activities [79]. It may also act as a potent inhibitor of CaOx crystal growth [80]. Calgranulin, an S100 protein, is present in the kidney and human urine and can inhibit growth of CaOx crystals, which is the major component of kidney stones. There are 3 monomers (A, B and C) all mapped to chromosome 1. The inhibitory properties of calgranulin may be due to its ability to bind to the crystal surface [81].

3.2. Promoters

On the cell surfaces of the kidney, cell debris, protein aggregates and other crystals may provide analogous site for nucleation. These nucleation sites may lower the SS required to initiate crystallisation and therefore promote CaOx crystallisation. Strong geometric similarities between the crystals of uric acid dihydrate and COM may promote overgrowth of one on the other, a process similar to the relationship between apatite and COM [8]. Evidence suggests that uric acid and CaP may promote heterogeneous nucleation. Another factor that may promote the formation and growth of intrarenal crystals is ionic calcium. Hypercalciuria can decrease inhibitor function and lead to crystallisation. Furthermore, cellular responses to newly formed crystals and factors that modulate these crystal-cell interactions could stimulate the initiation of an intrarenal stone [82].

4. Summary

Kidney stone formation is initiated by supersaturation of urinary salts and crystal retention in the urinary tract. Since the human nephron is a dynamic system, *in vitro* crystal behaviour is too simplistic a concept to entirely explain renal calculus formation. A complex medley of inhibitors and promoters are involved. Deficiency of inhibitors and/or an abundance of promoters in the urine are almost certain to predispose to stone disease.

Genetic linkage of some of these compounds may eventually lead to a greater understanding of familial stone disease, in addition to recognised dietary factors. The role of cell injury may be an even more important determinant in the promotion and progression of kidney stones. Perhaps the presence of the stone itself initiates an inflammatory response, leading to further epithelial disruption and amplification of stone formation. The regulation of inflammation may lead to attractive new therapeutic strategies for the management of stone disease.

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CME questions

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1. Spontaneous nucleation of crystals occurs:
   A. At the ion-activity product below the solubility product
   B. At the ion-activity product above the formation product
   C. At Zeta potential of +70
   D. In the metastable zone

2. Substrate for heterogeneous nucleation in calcium oxalate stone formation includes all of the following except:
   A. Calcium oxalate
   B. Uric acid
   C. Bacteria
   D. Epithelial cells

3. THP can loose inhibitory activity if urine has:
   A. High pH
   B. High concentration of calcium
   C. High concentration of sodium
   D. High concentration of hydrogen ions

4. The main force that inhibits calcium oxalate crystal aggregation is the repulsive electrostatic surface charge, known as:
   A. Alfa potential
   B. Beta potential
   C. Zeta potential
   D. Gamma potential

5. Following statement regarding citrate is false:
   A. It filters freely through the glomelurus
   B. Most of the circulating citrate is derived from endogenous oxidative metabolism
   C. Approximately 75% of the filtered citrate is reabsorbed in the distal convoluted tubule
   D. Most established effect of citrate in urine is to complex with calcium thereby reducing the concentration of calcium oxalate

6. All of the following have been suggested as possible inhibitors of stone formation EXCEPT:
   A. Matrix substance A
   B. Citrate
   C. Magnesium
   D. Pyrophosphate