Pathomechanisms of nephrolithiasis

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Abstract
Lithiasis continues to be an important factor in chronic renal disease, since it leads to chronic tubulo-interstitial nephritis, which is estimated to be involved in 15- 30% of cases of end stage chronic renal insufficiency. It is believed that in order for a stone to be formed, a solid phase needs to be first produced from microcrystals (the nucleus), which are formed from salts (and other substances) that are found dissolved in the urine (nucleosis of crystals). Afterwards, the crystals that constitute the core increase in size and link up with each other (incorporation). The main physiochemical factors that participate in the creation of the nucleus are the hypersaturation of urine, the lack of inhibitors of nucleosis and probably the organic substrate. In order for the increase in size and the incorporation of crystals to take place, hypersaturation, the lack of inhibitors, the organic substrate and the epitaxis, during which crystals of a substance are attached to the surface of other crystals of a different chemical structure (e.g. crystals of oxalic calcium onto crystals of uric acid) are needed. Various molecules have been found in urine, which modify to an important degree the adherence of crystals to the surface of epithelial cells. It also seems very likely that certain reactions of renal epithelial cells that follow the uptake of calcium oxalate monohydrate (COM) crystals are due to oxalate ions, which are released during the process of deconstruction of the intracellular crystals. From here, the crystals migrate in the median tissue, where an inflammatory reaction takes place and finally the crystals are destroyed. Macrophages gather in the crystals of the median tissue. The osteopontin which is related to the crystals acts as a chemotactic factor for the macrophages and therefore is perhaps involved in this process too. The uptake of crystals appears to be subjected to regulating mechanisms, as molecules which regulate the endocytosis of COM crystals, a process that is related to changes in the special components of the cytoskeleton, have been observed. In conclusion, the processes of adherence and of endocytosis promote the detention of crystals in the nephron, whilst intracellular deconstruction is an important factor of defence against the deposition of calcium in the kidney. Hippokratia 2013, 17, 2: 100-107

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Introduction

Twenty five years have already passed since the first application of extracorporeal lithotripsy in the management of renal stones (or more precisely urolithiasis). The memory of repeated surgical interventions, including those of large resections that frequently led to nephrectomy, hardly remains. However the mechanism of stone formation is still elusive and the pathogenesis of lithiasis is in effect unknown.

Lithiasis continues to be an important factor in chronic renal disease, since it leads to chronic tubulo-interstitial nephritis, which is estimated to be involved in 15- 30% of cases of end-stage chronic renal insufficiency.

Calcium oxalate is the most prevalent type of kidney stone disease in the United States and has been shown to occur in 70-80% of the kidney stone population[1]. The prevalence of recurrent calcium oxalate stones has progressively increased in untreated subjects, approaching a 50% recurrence rate over 10 years[2]. The lifetime risk for kidney stone disease currently exceeds 6-12% in the

general population[3]. In the final quarter of the twentieth century, the prevalence of kidney stone disease increased in both male and female and all ethnicities[3]. Although kidney stone nephrolithiasis is perceived as an acute illness, there has been growing evidence that nephrolithiasis is a systemic disorder that leads to end-stage renal disease[4]. It is also associated with an increased risk of hypertension[5], coronary artery disease[6], metabolic syndrome (MS)[7-9], and diabetes mellitus[8-13]. Nephrolithiasis without medical treatment is a recurrent illness with a prevalence of 50% over 10 years[2].

It is believed that in order for a stone to be formed, a solid phase needs to be first produced from microcrystals (the nucleus), which are formed from salts (and other substances) that are found dissolved in the urine (nucleosis of crystals). Afterwards, the crystals that constitute the core increase in size and link up with each other (incorporation). The main physiochemical factors that participate in the creation of the nucleus are hypersaturation of urine, lack

of inhibitors of nucleosis and probably type of the organic substrate. In order to increase in size and the incorporation of crystals to take place, hypersaturation, lack of inhibitors, organic substrate and epitaxis, during which crystals of a substance are attached to the surface of other crystals of different chemical structure (e.g. crystals of oxalic calcium onto crystals of uric acid) are needed.

Mechanisms of crystal formation in urine

A solution is considered saturated regarding a substance when it contains in dissolution its highest possible concentration. In other words, if one adds to the solution an additional quantity of this substance, it will precipitate and form crystals. When the salt density is small, the solution is hyposaturated and crystals cannot be formed. If crystals pre-exist in the solution, they decrease in size and could possibly dissolve (region of hyposaturation). If the density is increased, it will reach a point beyond which whatever quantity of salt is added it is impossible to remain in dissolution and it precipitates ('solubility product' or saturation level). If the density of the solvent increases even more, it will reach the 'formation product'.

The region between the solubility product and the formation product is called the transient or metastable region. There, despite the fact that the urine is saturated and the solvent is found at a density that is higher than what is needed in order to precipitate in a water solution, the crystalopoesis of salts and the formation of a nucleus does not happen. It is however possible that an increase in size and an incorporation of preformed crystals may occur. When the density of the solvent exceeds the formation product (a region of hypersaturation or an unstable region), precipitation of crystals and the formation of a nucleus (homogeneous nucleosis) takes place.

Moreover, in this region, a rapid increase in the size and incorporation of crystals occurs.

In clinical practice, hypersaturation can be the result of either an increase in the excretion of solvents in the urine (e.g. calcium, oxalates, cystine) or a reduction in the volume of the urine because of a decreased intake or an extra-renal loss of liquids. It is also influenced by the ionic activity of soluble salts, the pH, as well as the existence of soluble complex salts. Thus, an acidic pH favours the precipitation of uric salts, whilst the formation of inflammatory stones takes place in an alkaline pH.

Because urine contains a combination of anions, cations and macromolecular organic unions, it has the capacity to retain in dissolution ions that are found in higher concentration than the level of saturation. This capacity is due to the formation of stable solvents of complex salts that include charged anion-cation complexes, ions connected with macromolecules (e.g. mucoproteins) and anion-cation complexes connected with natural chemical substances, such as citric and pyrophosphate salts. This can explain why the precipitation of salts and the nucleosis of crystals in the transient region does not occur.

Therefore, in order for the crystalopoesis of salt and nucleus formation to occur it is not enough that the urine is hypersaturated in this salt, it is also necessary for its concentration to be above the formation product. Exceptions include uric acid, cystine and xanthine stones where hypersaturation is sufficient for their formation.

The calculation of the degree of saturation of a salt, e.g. of oxalic calcium in urine, is useful in the computation of the probability of stone creation. The activity product of a salt that is dissolved in ions C+ and A – is calculated by the product $K = \{C^+\} \cdot \{A^-\}$, where {} represents the chemical activity of the ion. This equation can become: $K = [C^+] \cdot Fc \cdot [A^-] \cdot fa$ where [ ] represents the concentration of free ions and f the activity constant. The concentration of free ions in a salt and the activity constant are possible to calculate for urine. In a number of specialized centres computer software is used for this[14].

The relative saturation ratio (RSR) is the ratio of the activity product divided by the solubility product. The solution is saturated when the RSR is equal to 1, hypersaturated when the RSR is higher than 1 and hyposaturated when it is lower than 1[15].

Prerequisits for the creation of a stone are hypersaturation, a nucleus and time in order to be formed. More than 200 components have been reported in the analyses of stones, with the main component being oxalic calcium.

Types of urinary stones

Calcium oxalate stones

Urine in the distal renal tubule is hypersaturated by oxalic and calcium ions which react between themselves forming crystals of dehydrated calcium oxalate (COD) (the commonest crystal in urine), and oxalic calcium monohydrated calcium oxalate (COM) (the commonest crystal in stones)[16].

The human serum oxalate concentration ranges between 1 and 5 Mm, however, due to water reabsorption in the kidney, its concentration is 100 times higher in the urine. At a physiologic pH, oxalate will form an insoluble salt with calcium. As the solubility of calcium oxalate in an aqueous solution is limited to approximately 5 mg/l at a pH of 7.0, assuming that normal urine volume ranges between 1 and 2 l/day and normal urinary oxalate excretion is less than 40 mg/day, normal urine is often supersaturated with calcium oxalate. However, under normal conditions, the blood is undersaturated in respect with calcium oxalate. As seen in patients with primary hyperoxaluria and renal insufficiency, when the serum oxalate concentration increases to above 30 μM, the blood becomes supersaturated with calcium oxalate. In the plasma, oxalate is not significantly bound to protein and is freely filtered by the kidneys. A recent study reported that urinary calcium is as important as urinary oxalate in raising calcium oxalate supersaturation[17].

The way in which these formed crystals are retained in the kidneys of sensitive individuals and form stones is unknown. Until now, data has shown that it is difficult for a crystal to grow to such a degree during its passage through the nephron in order to occlude its lumen. It appears that after the formation of the nucleus in the renal tubules, this nucleus is either incorporated with other crystals forming a mass capable of occluding the nephron, or it attaches to the epithelial cells of the tubules.

It is known that factors which influence the increase in size and the incorporation of crystals play a decisive role in to what extent a simple crystal or the incorporated crystals will attain such a mass that occlude the lumen. In an effort to explain the creation of stones, the degree of hypersaturation regarding calcium, oxalic, phosphates and uric acid, has been studied in detail, as well as the role of molecule-inhibitors of lithiasis, such as citric molecules, or macromolecules such as osteopontin.

Uric acid stones

Three major factors for the development of uric acid (UA) stones are low urine volume, acidic urine pH, and hyperuricosuria. However, abnormally acidic urine is the principal determinate in UA crystallization. The etiologic mechanisms for UA stone formation are diverse and include congenital, acquired and idiopathic causes. The most prevalent cause of UA nephrolithiasis is idiopathic. In its initial description, the term 'gouty diathesis' was coined. The clinical and biochemical presentation of idiopathic UA nephrolithiasis (IUAN) cannot be attributed to an inborn error of metabolism[18,19] or secondary causes such as chronic diarrhea[20], strenuous physical exercise[21], and a high purine diet[22].

The metabolic defect suspected for low urinary pH in UA stone formation was described almost four decades ago. Defective ammoniagenesis or excretion was attributed as a possible pathogenetic mechanism. Initial studies showing abnormalities in glutamine metabolism, which resulted in the impaired conversion of glutamine to α-ketoglutarate and consequently resulted in reduced renal ammonium (NH) excretion, were not supported by further investigation[23]. Mechanistic studies, however, have shown that the two major factors responsible for abnormally low urine pH are a combination of defective NH excretion and increased net acid excretion.

In the majority of kidney stones, CaOx is the main constituent and CaP is present in amounts ranging from 1% to 10%[24]. When CaP becomes the main constituent (>50%) of stones, the stones are called CaP stones, and patients who form CaP stones are referred to as CaP stone formers. CaP is present in urinary stones as either apatite (the principal constituent of bones and teeth) or brushite (calcium monohydrogen phosphate).

Calcium phosphate stones

It is known that 20% of stones contain hydroxyapatite (HA), and that HA crystals adhere rapidly to cellular surfaces in a linear relationship with the concentration of COM crystals. This adherence has been suspended under laboratory conditions with various polyanions such as heparin, polysulphate pentozane, polyaspartate, polyglutamate and other molecules that have been detected in the urine of the renal tubules, such as the sulphate chondrotines A and B, sulphate heparine, citric, nephrocalcine and osteopontine. Polykations cetylpyridinium chloride and cationized ferritin, the cationic dyes acian blue, polyethyleneimine and brilliand blue R, as well as the lectin T vulgaris also suspend the adherence of HA crystals.

It appears that the anions that inhibit the adherence of crystals act via the surface of those crystals, whilst the lectines and the cations exert their action on the epithelial cells.

Incubation of these cells with neuroaminidase resulted in the suspension of adherence, implying that the existing molecules that connect HA crystals to the surface of the cell membrane, have as their base sialic acid, it is also for this reason that they are blocked by special lectines.

Brushite is a unique form of CaP, which in certain patients can form into large symptomatic stones. Treatment of brushite stones can be difficult since the stones are resistant to shock wave and ultrasonic lithotripsy, are often require ballistic fragmentation. Patients suffering from brushite stone disease are less likely to be rendered stone-free after surgical intervention and often experience stone recurrence despite maximal medical intervention. Studies have demonstrated an association between brushite stone disease and shock wave lithotripsy (SWL) treatment. Some have theorized that many brushite stone formers started as routine calcium oxalate (CaOx) stone formers who sustained an injury to the nephron (such as SWL). The injury to the nephron leads to failure of urine acidification and eventual brushite stone formation[25].

Sturvite (magnesium ammonium phosphate) stones

Sturvite stones account for 15% of renal calculi. They are associated with chronic urinary tract infection (UTI) with gram-negative rods capable of splitting urea into ammonium, which combines with phosphate and magnesium. Usual organisms include *Proteus, Pseudomonas,* and *Klebsiella species. Escherichia coli* is not capable of splitting urea and, therefore, is not associated with sturvite stones. Urine pH is typically greater than 7.

Underlying anatomical abnormalities that predispose patients to recurrent kidney infections should be sought and corrected. UTI does not resolve until stone is removed entirely.

Inhibitors of stone formation

Various studies have proposed another mechanism by which crystals are retained in the kidney: It has been shown that COM crystals connect rapidly with the surface of renal epithelial cells, which endocytose them[26]. The surface of these COM crystals behaves as if positively charged, whilst the lumenal surface of the epithelial cells of the tubules behaves as if negatively charged. Therefore this adherence is due to reactions of electrical charge. Anionic molecules have been found on the surface of epithelial cells and act as COM crystal receptors[27,28]. However, in the urine of the tubules anions in dissolution exist, adhered to the surface of crystals, preventing them connecting with the epithelial cells[29].

Changes in the quantity and the structure of specialized anionic molecules that are expressed on the surface of the epithelial cells of the tubules or those that are found in dissolution in the urine influence the adherence of crystals to cells, therefore participating in urolithiasis.

In order to study more clearly the reaction between renal epithelial cells and crystals, cellular cultures of renal epithelial cells of the ape (BSC-1-line) have been de-

veloped, to which radio labelled COM crystals have been added. The adherence of crystals to the surface of epithelial cells is observed within 15 seconds and is completed within 30 seconds. The addition of larger numbers of crystals results in a linear correlation with the connection of these crystals. The same phenomenon of adherence of cells has also been observed in cultures of renal epithelial cells of dogs (MDCK line) and in cultures of fibroblasts[26].

It has also been shown that a large number of cells hold on to COM crystals with a preferential rate 10 times greater than to brushite (phosphoric calcium). Consequently, the adherence of COM crystals in the cultures of renal epithelial cells is fast and specialized, thus showing the existence of kinship between these crystals and the surface of epithelial cells.

Molecules inhibiting lithiasis in the urine

Various molecules have been found in urine, which modify to an important degree the adherence of crystals to the surface of epithelial cells.

Heparin is known to powerfully inhibit the increase of COM crystals, behaving as a model polyanion that almost completely suspends the capacity of crystals to attach themselves to epithelial cells. Heparin also inhibits the capacity of COM crystals to adhere in a culture of fibroblasts, showing that its inhibiting action is not unique only to renal epithelial cells. Though heparin has not been found in urine, glycosaminoglycanes (GAGs) are present and include sulphate roots[30].

Sulphate chondroitine A and B, heparin sulphate and hyaluronic acid are also known to inhibit the adherence of COM crystals even if somewhat less effectively than heparin.

There are also other soluble anions in the urine of renal tubules, which decrease the adherence capacity of COM crystals to the surface of epithelial cells. Polyanionic citric prevents the adherence of COM crystals, when they are found in concentrations roughly equal to those that are physiologically found in urine (250m M). Sulphate pentozane, a synthetic anion which exerts a powerful inhibiting action on the increase of the size of crystals and is excreted in the urine following oral administration, also prevents the adherence of COM crystals. These facts may perhaps lead to a new generation of medications for the treatment of nephrolithiasis[31].

Numerous glycoproteins found in urine have also been examined. They appear to play a role in nephrolithiasis, and include nephrocalcine, uropontine (a potent inhibitor of the increase in size[32] of COM crystals) and the Tamm-Horsfal protein (THP), which prevents the incorporation of these crystals. Nephrocalcine and uropontine, in concentrations similar to those found in human urine, show a powerful inhibiting action towards the adherence of COM crystals by something which has not been observed with THP[32,33]. These anions suspend the adherence of crystals by covering their surface.

In summary, two populations of polyanions, one which is found anchored to the surface of the cellular membrane in the lumen of tubules and the other which is found free within the urine of tubules, can be considered as competitors of crystals for the surface. Changes either in the quantity or in the composition or in the quality of each one of these populations of anions could disturb the balance or determine the final fate of crystals which form a nucleus within the urine of the tubules.

The adherence of these crystals may be strengthened either by the increase in the number of anionic molecules on the surface receptors or by a reduction in the quantity of the special anions in the urine of tubules or by both.

Anions on the surface of tubular epithelium

Since soluble polyanions that have a very close relationship with COM crystals and prevent their adherence to epithelial cells exist, there must also exist similar anions in the cellular membrane that compete with those in the urine.

In cell cultures of renal epithelial cells to which various molecules with a positive charge have been added, it has been shown that these molecules connect with anions on the surface of the cellular membrane, thus preventing crystals adhering to it. When cationic feritine was added to these cell cultures before the addition of crystals, the adherence of these crystals to the cells was almost completely suspended[27]. Chloride setilpyridinium, a cationic molecule often used in solutions for the precipitation of polyanions such as the glycosaminoglycans, also prevents the adherence of COM crystals. Similarly, various polykationic dyes such as polyethyleneimine, asian blue, blue dextran, briliant blue R, acridine orange, and safranine have also been tried[34]. All these dyes also prevented the adherence of COM crystals even if they had to be used in higher concentrations than the two previous polykations. It appears that interference in the adhesion of COM crystals is the result of something more than just electric charge alone, since the other cations that have been utilised have had no effect. Each one of the above effective polykations connected mostly with the epithelial cells instead of the surface of the COM crystals, thus implying that the surfaces of the epithelial cells express anions related to the adherence of the COM crystals.

An important group of anions present on the surface of the cellular membrane of renal epithelial cells are various glycosidic unions that are blocked by special lectines.

Studies performed with special lectines connected with glycosides of the surface of epithelial cells, such as the lectin of triticum vulgaris (connectin from the seeds of wheat), showed that this inhibited the adherence of COM crystals. Two other lectines - of the hemocyanin type that are connected with glycosides which contain sialic acid - have also been shown to have the same action. Two lectines known to be non-connected with sialic acid, concanavaline and Solanum Tuberosum have not shown any action.

Since the lectines and the polykations that inhibit the adherence of COM crystals are connected with the glycoproteins expressed on the surface of epithelial cells that contain sialic acid, the action of specific enzymes on these glycoproteins has been studied.

Incubation of epithelial cells with two proteases, proteinase K and trypsine, resulted in a partial suspension of

the adherence of COM crystals, whilst a much greater inhibition was observed in the incubation of cultures with neuraminidase. Incubation of these cultures with heparin sulphatase or chondroitinase A, B, C did not show any such action regarding the adherence of crystals. These results demonstrate that COM crystals have a connection with the glycoproteines that contain sialic acid on the surface of renal epithelial cells (N - aketyloneuraminic acid).

Pathomechanisms of renal stone-induced renal damage

Oxalate is an end product of metabolism that is excreted by the kidney[35]. This dicarboxylate had been considered to be an inert metabolic by-product; however, several studies[36-42] demonstrated that oxalate exposure elicits a variety of changes in renal epithelial cells, ranging from increased DNA synthesis and an induction of ''immediate early genes'' to increased membrane permeability and cell death. The manner by which oxalate triggers these changes remains unclear. The observation that oxalate can increase free radical production in renal epithelial cells, coupled with the well-documented role for free radicals as intracellular signals, suggests that superoxide or another reactive oxygen species (ROS) may mediate oxalate actions. Indeed, antioxidant agents block both free radical generation and the changes in cell viability produced by oxalate exposure[38].

Various studies have implied that perhaps the lipids of the cellular membrane play a role in the adherence of COM crystals[36-39]. In studies where incubation of epithelial cells of connective tubules of mice with liposomes took place, so that the cellular membrane was enriched with phosphatidyloserine (PS), the adherence of COM crystals was increased. This result was neutralised by the addition of anexine, a factor that connects to PS. Physiologically, PS has been found only to be present on the internal surface of the cellular membrane folds, apart from cases of cell damage or death via apoptosis[40-42]. It could be extrapolated from this that increased adherence of COM crystals would be observed in dead or wounded cells. The viscosity of the cellular membrane is influenced substantially by the constitution of the lipids. As long as the viscosity increases, the adherence of COM crystals also increases. These two observations show that the constitution of the lipids of the cell membrane can influence the adherence of COM crystals via independent mechanisms.

It has been shown that both the ions of oxalate calcium and COM crystals exert a toxic action on renal epithelial cells[43]. In vitro and in vivo studies have demonstrated that dead cells and the products of cellular inflammation promote the formation of COM crystals at lower levels of hypersaturation and that they also contribute to the detention of crystals inside the renal tubules[44,45]. Consequently, it appears that renal inflammation is a predisposing factor to the nephrolithiasis of oxalate calcium[46]. All evidence points to the fact that the inflammation of cells is implicated in the formation of stones. However, it has not been clarified if their damage is caused by the oxalic ions or the COM crystals.

Renal tubular damage and death have been observed in mice who have become hyperoxaluric after oral administration of ethylenoglycol, which causes the formation of COM crystals in the lumen of tubules. Oxalates in low concentrations (80-320 mM) promote the growth of cells but in higher concentrations (400- 1600 mM) they can cause cell death[47].

Jointly, these studies show that the cellular reaction that occurs in COM crystals or in the oxalate ions depends primarily on the concentration of ions and on the cellular type.

It is also seems very likely that certain reactions of renal epithelial cells that follow the uptake of COM crystals are due to oxalate ions, which are released during the process of deconstruction of the intracellular crystals.

Cell death can occur either by necrosis or by apoptosis and the way in which this happens has great significance[48].

The main criteria that are used to determine the morphological alterations seen in apoptosis are the condensation of chromatin in the nucleus, its breaking into pieces and its displacement to the periphery of the nuclear membrane[49].

During the process of apoptosis cells shrink, cellular membrane maintains its integrity, PS from the interior of the cytoplasmic membrane appears on its surface chromatin in the nucleus condensates and DNA is split into 180 - 200 pieces[50,51].

During the process of necrosis cell expands, cenotopes grow, mitochondria appear oedematous, cytoplasmic membranes undergo specific alterations, and sacculations may form on the surface of those membranes. In any case the cells which undergo necrosis do not express an increase of PS on their surface or a condensation of chromatin or segmentation of their DNA.

Finally, cells that undergo apoptosis are detached from their basic membrane and are sliced into smaller particles, which are phagocytosed by the neighbouring cells, while necrotic cells undergo rupture pouring their contents into the neighbouring region. Consequently, necrosis of cells can cause an inflammatory reaction, while cell death via apoptosis is less likely to do so.

The apoptosis of renal epithelial cells that is observed in response to the action of oxalates probably plays a very important role in nephrolithiasis. During the changes observed in apoptosis, the anexin appears on the surface of cells connected with PS. Groups of negatively charged molecules of PS attract calcium and they can function as areas of adherence for COM crystals[52]. Moreover, such groups of negatively charged molecules on the surface of apoptotic cells and the products of deconstruction of cellular membranes can promote the heterogeneous nucleosis of calcified salts, such as calcium phosphate and oxalate.

The exposition of the denuded basic membrane after the detachment of the cells can have great significance in the pathogenesis of nephrolithiasis. Studies with both simple and electron microscope have clearly shown that COM crystals are attached to the basic membrane. Simultaneously, components of the extracellular matrix, such as integrines, have a high affinity to oxalate calcium crystals or to macromolecules that are related to COM crystals, such as osteopontin[53,54].

Studies have shown that oxalate ions and COM crystals activate the renal epithelial cells. The response of the

cells depends on the quantity of oxalates and the presence or the absence of COM crystals. Under normal concentrations of oxalates, crystals are formed occasionally and they are either removed in the urine as crystalluria or they are endocytosed by the epithelial cells[55]. Crystals that undergo endocytosis are deconstructed by lysosomal enzymes or they are exocytosed in the lateralateral region of the epithelial cells. From here, crystals migrate in the median tissue, where an inflammatory reaction takes place and finally they are destroyed. Macrophages gather in the crystals of the median tissue. The osteopontin which is related to the crystals acts as a chemotactic factor for the macrophages and therefore might be also involved in this process.

Moderately higher levels of oxalates inside the metastable zone cause the excretion of modifiers of crystallization[53,54]. Long-lasting exposure to high levels of oxalates causes cell destruction. The production of modifiers maybe interrupted. The cellular membranes become sensitive to the adherence of COM crystals. Epithelial cells with or without the endocytosed attached crystals are finally extracted from their basic membrane. Sacculations which are found in the cellular membrane as a result of cellular deconstruction promote the heterogeneous nucleosis of crystals, even at lower levels of hypersaturation, also contributing to the incorporation of crystals. Crystals are retained by the kidneys by incorporation with other crystals and products of cellular deconstruction, as well as by adherence to the exposed denuded basic membrane. When the withheld crystals are exposed to the urine in the renal pelvis, they act as a "nest" for the growth of stones.

Functional changes of epithelial cells

The uptake of crystals appears to be subjected to regulating mechanisms, as molecules which regulate the endocytosis of COM crystals, a process that is related to changes in the special components of the cytoskeleton, have been observed. Within one to three hours following the reaction of COM crystals with the renal epithelial cells[56], microvilli of actin are formed precisely under the attached crystals. It appears that this actin plays some role in endocytosis of crystals.

Mitogenous factors such as epidermal growth factor, nucleotide adenosine diphosphate, bovine serum at a high concentration >10% and cultural media with low K^+ concentration increase the uptake of crystals, while heparin, transforming growth factor beta 2, tetrapeptide arginine - glycine - aspartic acid - serine (RGDS) and extracellular protein fibronectin which contains RGD lead to a reduction in crystal uptake.

Concerning the endocytosis of crystals, the action of THP, which is found in abundance in urine, has also been studied, but its role remains almost unknown. THP which is isolated from human urine decreases the endocytosis of COM crystals by tenfold compared to that which usually exists in the urine[57]. This suspension of endocytosis and of COM crystals occurs due to the action of THP on the surface of the cells and not due to an action on the surface of the crystals. The endocytosis of crystals happens with the mediation of actine microvilli of the cytoskeleton and

it is regulated by various molecules that appear to act independently. Moreover, the capacity of THP to block the uptake of COM crystals in the upper spiral tubules might play a protective role in nephrolithiasis. The combination of these factors in the reaction between crystals and cells probably determines whether the crystals will be withheld in the nephron or will be removed in the urine.

These crystals, when added to cellular cultures, act as a stimulus for cellular proliferation and incorporation of [3H] thymidine into DNA.

A remarkable observation has been reported when stimulation of the composition of DNA from two mitogenous factors, COM crystals and bovine serum at 10% takes place. It was noticed that a delay of eight hours in DNA synthesis occurred following the action of those factors. This delay perhaps represents the time required for the processes that happen during the adherence and endocytosis of crystals and the subsequent production of signals which stimulate cellular proliferation[58].

Various cellular functions appear to change with the uptake of crystals. Eight hours following the addition of COM crystals to cellular cultures of apes, kytokeratin 8, a component of medium sized fibrils of the cytoskeleton, appears to be distributed in every cell of cellular culture not only in those that have endocytosed crystals. On the contrary, cells that were incubated with COM crystals and monoclonal antibodies in tubuline did not present morphological alterations. Contrary to the changes observed in the microvilli of actin, changes seen in the network of cytokeratin did not appear to relate to the uptake of crystals. In addition, all cells of the culture showed a rearrangement of microvilli of cytokeratin 8 after incubation with COM crystals. This shows that a communication between cells does exist, which potentially produces an autocrine factor that is at present unknown.

Genes changes

The incubation of renal epithelial cells with COM crystals is also related to changes that are observed in the expression of certain genes.

Premature genes are produced after hours of incubation with crystals and include early growth response 1, that is an activator of copying; Nurr-77 the protein connected with DNA; c-jum, a proto-oncogene that manifests the activity of a precocious gene; and c-myc that acts as a copying factor[59].

In addition, genes have been studied which contribute to the formation and the deconstruction of the extracellular matrix (ECM) through the activation of plasminogen, implied by intermediate fibrosis which is a prime and constant finding in the biopsy samples of kidneys of patients with hyperoxaluria and deposition of crystals. After an incubation period of 2-6 hours with COM crystals, a spectacular increase was observed in the expression of the gene that codes for rapid action inhibitor of the activator of plasminogen 1 (PAI-1). However, expression of the gene which codes for the activator of plasminogen in urine did not change, and the activator of plasminogen in tissue was not expressed at all in these cells. Increased

expression of PAI-1 without simultaneous change in the activator of plasminogen in urine could lead to a decreased production of plasmin, producing an increase and accumulation of the proteins of the ECM and finally leading to interstitial fibrosis of the kidney[60,61].

In cell cultures of BSC-1 cells, the Platelet-Derived Growth Factor (PDGF) A chain mRNA is normally expressed in controlled conditions. Nevertheless, after an incubation period of 2-6 hours with COM crystals, an important stimulation in the expression of the above-mentioned factor is observed; the gene that codes for the PDGF-B chain is expressed but its production is not stimulated. The extraordinary expression of PDGF-A chain mRNA may produce an increase in the production of this paracrine growth factor, which is known to stimulate the proliferation of fibroblasts in kidney and to promote the production of collagen[62].

One hour after incubation with COM crystals, a copy for connective tissue growth factor is produced, a protein that is rich in cysteine and has immunological activities as well as PDGF actions; its production continues for more than 24 hours[63].

In cell culture of BSC-1 cells, under controlled conditions, many genes were expressed. Theses genes regulate the adherence of COM crystals to cells and the production of ECM. These genes code for proteins such as laminin, collagen, fibronectin, and transforming growth factors beta 1 and 2. Genes that code for protease collagenase stromolysine, which deconstruct the components of the ECM, were not expressed, unlike the genes that code for acidic or basic growth factors of fibroblasts, that were expressed and constitute important regulators of the ECM.

The interstitial scarring that is observed in the kidneys of patients with primary or secondary hyperoxaluria, could be attributed to a reaction of epithelial cells to the COM crystals with a simultaneous increase in expression of specific genes, which promote the production of proteins that accumulate in the ECM and lead to fibrosis.

The uptake of crystals from tubular cells can be considered to constitute part of a positive feed-back mechanism that favours the adherence of additional crystals, implied by studies with electron microscope, which reveal that attached COM crystals on the surface of BSC-I cells behave as places of incorporation for other crystals[55]. Increased adherence of exogenous crystals to regions of the cytoplasmic membrane where there are endocytosed crystals was observed at least 24 hours following the initial reaction between cells-crystals.

However, cells seem to have a metabolic mechanism via which they deconstruct crystals; these crystals following endocytosis appear to be destroyed by lysosomes. The process of deconstruction of crystals is relatively slow and takes 5 – 7 weeks to be carried out completely. During this period of time many cells which contain crystals are being continually stock piled. There is evidence that similar reactions and processes also take place in the intact nephron.

In conclusion, the processes of adherence and of endocytosis promote the detention of crystals in the nephron, whilst intracellular deconstruction is an important factor of defence against the deposition of calcium in the kidney.

Conflict of Interest

Author declares that no funding was received for this research and therefore there is no conflict of interest.

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