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Lithogenic Modulator Mechanisms: Current and Future Directions

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Abstract

This review aimed to focus on various modulators involved in the crystal progression and inhibition. Kidney stones are a crystal concretion that is usually formed inside the kidneys. Renal calculi and its pathogenesis are remaining unclear. Apart from the fact that salts in the urine are important, the abundance of these salts themselves does not always lead to the formation of stones. Glycoprotein and glycosaminoglycans, membrane lipids enhanced nucleation-promoting activity are the macromolecules involved in inhibitory activities. The most studied inhibitory proteins are Nephrocalcin, Tamm-Horsfall protein, Osteoponin, Urinary Prothrombin Fragment 1 and Bikunin. This review critically discussed about the molecular mechanism, pathogenesis and promoters or inhibitors of renal or calcium oxalate stone formation.

Key words: Kidney stones, crystal progression, glycoprotein, nephrocalcin, urinary prothrombin fragment-1, osteopontin

INTRODUCTION

Kidney stones have plagued humans for thousands of years. Many researchers are trying to evaluate the structure of the CaOx calculi formation. With its multifactor etiology and high rate of recurrence, UTI infections are urges a medical challenge (Aggarwal KP., 2013). Therefore, there is an urgent need to prevent this disease and its recurrence. Physiochemical mechanisms of stone formation are through aggregation, growth, coagulation, and the interaction of various transformers in urine. The lifespan of urinary tract infections is 12% for men and 6% for women and the incidence increases, leading to about 12,000 hospitalizations each year (BAUS, 2014). Calcium-derived stones, both in oxalate and in phosphate form, are found in most of the cases, while infectious lithiasis remains a major cause of this condition in developing countries. The metabolic, anatomical and physiopathological methods are involved in the formation of calcium stones.

It needs to understand the pathophysiology of nephrolithiasis due to lack of proper identification yet (Bao and Wei, 2012). However, risk factors for kidney stones can be internal (such as age and gender) or external (such as diet and climate). According to BAUS (2014) the most common cause of abnormal kidney stones formation or abnormality. Small lesions on the kidney papilla are the main cause of the formation of monohydrate calcium oxalate stones, while the presence of these lesions and their characteristics will determine in most cases the final morphology of renal calculus.

At the stage of excess concentration of these ions crystallization leads to its saturation component. The crystals in the urine are highly concentrated and then stick to urothelium which can increase subsequent stone growth (Dawson and Thomson, 2012), and the formation of stone formation occurs between chemicals and development of kidney stones. Urine dilution can dissolve of crystallization of stone forming salts and contrast to it dehydration may lead to the development of kidney stones.

Calcium kidney stones are an important urological and physiological pathological condition that affects a high percentage of people during their lifetime. The formation of applied crystallization sites with the ability to urinate through urine is a considerable factor in calculus formation. Blood and urine tests should be ordered when you need to know blood levels of calcium, phosphorus, sodium, potassium, chlorine, magnesium, urea, creatinine and uric acid. In a 24-hour urine test, it is recommended to determine urea, creatinine, uric acid, oxalate, citrate and magnesium levels, and to determine the volume of diuresis, pH and urine density. The kidneys regulate phosphorus-calcium metabolism by releasing and reabsorbing calcium and phosphorus and also in the1.25 OH vitamin D and iPTH metabolism.

Hypercalciuria is the most commonly seen in metabolic dysfunctions, in 35-65% of patients with stones. The link to calcium levels and Randall's plaques and another factor to consider is genetic and cellular relationships are related to urinary calcium levels. Hypercalciuria is defined, according to various studies, as urination of more than 260 mg of calcium in 24 hours (or 4 mg of calcium kg / day). The types of hypercalciurea (during fasting calcium / creatinine ration >0.11 and >0.22 after intake of calcium) called as absorbent type; if the cause is due to failure of renal reabsorption called as excretory hypercalciurea and if it is from bone marrow, especially in relation to primary hyperparathyroidism called as Reabsorptive hypercalciurea.

Hyperoxylurea in intestine is a common disease, and the results are due to mal absorption, the causative factor for this infection is *Oxalobacter formigenes* and leads to increased oxalate absorption at intestine and bariatric surgery is also one the key cause of calculi with oxalate.

Another condition of lesser citrate excretion i.e 320mg/day in urine called as hypocitraturia.

Urinary citrate is regenerated under conditions of acidosis, which results in decreased urine output. If the urination with more than 600mg/day uric acid termed as hyperuricosuria and its cumulated levels in urine increases the stones formation by oxalate nucleation. The main components of a stone matrix resulted by its total dry weight approximately 2-3% and contain common macromolecules present in the urine. They are described by Boyce as 64% protein, 9.6% nonamino sugar, 5% hexosamine as glucosamine, 10% water bound, and remainder as inorganic ash. Although not found by Boyce, lipids are the other important component of stome metrix (Aggarwal KP., 2013).

Five major defects work together to improve cholesterol cholelithogenesis, including (i) Lith genes and genetic factors; (ii) hepatic hypersecretion of biliary cholesterol, including cholesterol-supersaturated gallbladder bile, i.e., cholesterol saturation index (CSI); (iii) rapid cholesterol nucleation and crystallization and rapid growth of solid cholesterol crystals; (iv) gallbladder motility dysfunction, which leads to the breakdown of inactive gallbladder and by mucin hypersecretion and gel formation, ultimately improving the formation of biliary sludge, i.e., precursor gallstones; (v) intestinal factors such as increased delivery of cholesterol that absorbs small intestine to the liver through biliary hypersecretion, changes in gut microbiota, and slow bowel movements. These defects work together to significantly reduce cholesterol solubility in the gallbladder, greatly promote cholesterol nucleation and crystallization, and accelerate the growth and cohesion of solid crystals such as cholesterol monohydrate crystals to form microlithiasis and ultimately larger gallstones (Wang DQ., 2017).

Molecular mechanism of renal stone formation?

Steps involved in the formation of Kidney stones:

The kidney stone formation in the three broad conceptual categories requires: Excessive concentration of solutes in excess of their solubility in the urine.

- Imbalance of modifiers (promoters and inhibitors) and crystallization in the urine.
- Epithelial abnormalities that allow attachment and subsequent growth of these crystals in to stone
- Above the factors act in concert and eventuating in the formation of the kidney stones (Moe et al., 2002).
- Moreover, calcium oxalate (Caox) crystals, the main constituent of human urinary calculi may adhere in the plasma membrane of epithelial cells by a specific manner and followed by endocytosis of the crystals resulting to cell damage or death.

Damaged cells exhibit a proliferation response and increase the fibrogentic synthesis; it is a substance promoting additional stimulus for crystal growth (Mirian et al., 2010). Calcium stone formation involves different phase of increasing accumulation of Caox and cap-nucleation, crystal growth, crystal aggregation and crystal retention.The physico-chemical analysis describes stone formation as a supersaturated solution in which homogenous or heterogeneous nucleation can lead to initiation of crystal formation, which can then aggregate and growth (Bhuskute et al., 2009).

Supersaturation:

Urinary supersurturation is the driving force behind crystal formation in the kidneys. Since formation of crystalline particles must obviously start from supersaturation, supersaturation is undoubtedly essential for stone formation. Indeed, stone formers tend to excrete urine that is more supersaturated than that of non-stone formers. 9–12 However, supersaturation values overlap widely,10 and people who have never formed a stone may nonetheless pass highly supersaturated urine.11 Humans normally excrete millions of urinary crystals daily, indicating at least transient development of supersaturation.

Nucleation

Nucleation is the process by which free ions in solution associate into microscopic particles. Crystallization can occur in solution micro-environments, such as may be present in certain points in the nephron, as well as on surfaces, such as those of cells and on extracellular matrix. There is considerable dispute about the importance of free solution crystallization versus crystallization at other sites, in renal tubules or on bladder walls, on normal or damaged cells, on areas denuded of cells by certain forms of injury, or at interstitial sites (Evan AP., 2003).

In vitro and *in vivo* studies have shown that renal tubular cell injury can promote crystallization of CaOx crystals by providing substances for their heterogeneous nucleation. In vitro cell degradation following renal tubular cell injury produces numerous membrane vesicles, which have been shown to be good nucleators of calcium crystals. In vivo crystals observed in the renal tubules of hyperoxaluric rats are always associated with cellular degradation products (Fasano JM., 2001).

Crystal Growth:

Once a crystal nucleus has achieved a critical size and relative supersaturation remains above one, the overall free energy is decreased by adding new crystal components to the nucleus. This process is called crystal growth. Crystal growth is one of the prerequisites for particle formation and thus for stone formation. In each step of stone formation, crystal growth and aggregation have important functions. Honda et al. reported that the crystal surface binding substance, which is found in CaOx crystals generated from whole human urine, is a strong inhibitor of CaOx crystal growth and contains proteins like human serum albumin, retinol binding protein, transferrin, Tamm-Horsfall glycoprotein, and prothrombin (Honda M., 1997)

Since the rate of CaOx crystal growth is low and the transit time of tubular fluid through the kidney amounts to only several minutes, it has been calculated that the probability of a single particle achieving a pathophysiologically relevant size by the process of crystal growth alone is extremely low, even if growth proceeds at an uninhibited rate of 2 mm per minute. The inhibitory effect of fibronectin (FN), a multifunctional a2-glycoprotein distributed throughout the extracellular matrix and body fluids, on CaOx crystal growth is small, considering the quantity normally excreted. FN at a

concentration of 0.5 mg/mL causes only 9.9% inhibition of CaOx crystal growth.

Aggregation

Aggregation is a process by which there is agglomeration of crystals that form in free solution into larger multicomponent particles. It may also encompass the phenomenon of secondary nucleation of new crystals on the surface of those already formed. The structure of stones suggests that one or other of these processes must occur for the stone to grow to a clinically significant size (Gower LB ., 2010). Kidney stones can be thought of as being similar to concrete, a mixture of a binding agent (cement), and particulates such as sand, pebbles, or glass. Stones are an aggregation of crystals and an organic matrix, the latter serving as the binding agent. The organic matrix contains proteins, lipids, polysaccharides, and other cell-derived material.

Crystal aggregation is promoted by viscous binding, implying that crystal-foreign compounds with multiple binding sites, such as abnormally self-aggregating Tamm-Horsfall glycoprotein or other macromolecules, attach to crystal surfaces and act as a kind of glue (Doyle IR., 1995). The inhibitory effect of fibronectin on CaOx crystal aggregation was found to be 47.7% at the 0.5 mg/mL physiological concentration of excreted fibronectin.

Crystal growth

Growth of microscopic crystals is accomplished by movement of ions out of solution onto the growing crystal. While some growth of nuclear crystals must occur by movement of ions from solution, this is clearly a limited process, as giant single crystals of stone constituents are not generally observed. It is more likely that stone growth is accomplished through aggregation of preformed crystals or secondary nucleation of crystal on the matrix coated surface of another. It has been proposed that the growth of these microscopic crystals to the extent that they can be retained in the kidney on the basis of size alone cannot occur without aggregation or attachment to specific intrarenal structures.

Retention:

Retention Crystal retention can be caused by the association of crystals with the epithelial cells lining. Urolithiasis requires formation of crystals followed by their retention and accumulation in the kidney. Another process that may lead to stone formation is crystal retention. i.e., crystal precipitation, growth, and aggregation, which results in urinary stone formation, if the nucleated crystals were flushed out by urinary flow. Retention might also depend on the composition of the renal tubular epithelial cell surface (Verkoelon et al., 2006).

Stone matrix protein modulators of crystallization in nephrolithiasis

The organic matrix of most urinary stones holds 2-3% of its dry weight, rare matrix stones have a matrix content of 65%. The organic matrix of urinary stones contains lipids, GAG carbohydrates, and proteins, containing proteins that contain about 64% of the matrix. Urinary trefoil factor 1 (TFF1) can abolish the growth of calcium oxalate crystals coagulation and bikunin (α-1-microglobulin) can prevent crystallization of stones, growth and coagulation. However, some stone modulators proteins have shown confusing an effect on stone formation due to Tamm-Horsfall (uromodulin) protein to promote crystal synthesis but another side inhibits the growth of calcium oxalate crystals. There are 13 proteins were founds in stones are human serum albumin (HSA), α1-acid glycoprotein (α1-GP), α1-microglobulin (α1-M), immunoglobulins (Igs), apolipoprotein A1 (apo-A1), transferrin (Tr), α1-antitrypsin (α1-T), retinol-binding proteins (RBP) and renal lithostathine (RL). B2-microglobulin (β2-M) was present only in calcium oxalate and uric acid stones.

Lipids

This layer of phospholipids promotes variety of calcium type crystals formation and forms living matrix for growing calcifications. Mainly struvite, uric acid, CaOx, and CaP, contain lipids. Phospholipids account for 8.6% of total lipid, which also represents approximately 10.25% of the stone matrix. The various phospholipids and glycolipids identified include sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanolamine (PE), cardiolipin (CL), and phosphatidylserine (PS) levels in all stone matrices. In some cases, the stone matrix contains phosphatidylinositol (PI), lyso-PC, lysophosphatidic acid (PA) and lyso-PE. In all glycolipids crystals include gangliosides, sphingosine and glucocerebrosides (Khan S. R., 2002).

Glycosaminoglycans (GAGs)

GAGs can report up to 20% of matrix weight. They may do some work on stone construction. The urinary GAGs usually contain 55% chondroitin sulphate (CS), 20% heparan sulphate (HS), 11% CS sulphated low, and 4-10% hyaluronic acid (HA) during normal condition. Urinary GAGs come from two sources. The first source of GAG urination is serum, which is filtered through the kidneys and enters the urine. The electrophoretic forms of GAG in urine are similar to those present in serum; in addition, GAG excretion of urine increases with increasing concentration of GAG in serum (Poon NW., 2012). Among CS, GAG is most abundant in urine, although it is present in small amounts of magnesium ammonium phosphate and apatite stones not found in CaOx crystals. Therefore, a standard measure of total urinary GAGs is likely to be of little benefit in the diagnosis and management of nephrolithiasis (Ryuichi M., 2016).

Protein

The stone matrix has been shown to contain an increasing range of proteins. Several proteins in urine subjected to intensive research due to its occurrence in crystals or because they have been separated from urine and which showed influence on crystallization on CaOx. Many studies on Tamm-Horsfall glycoprotein because it has the longest interaction with history and crystals, has been extensively tested (Kolbach-Mandel AM., 2017).

Tamm-Horsfall (THP) protein

Tamm Horsfall glycoprotein (THP), enjoys a unique position, perhaps high protein content in human urine and also the initiation of matrix protein formation. THP is usually found in crystals, regardless of the crystal components. THP is not present in CaOx crystals from all

urine, which may appear to indicate that THP binds only weakly, in that case, to CaOx crystals. It was long been accepted the crystal inhibitors might responsibly in crystal areas, it can expected that the THP is a negative barrier to CaOx crystallization at least in urine and also acts as a crystal inhibitor (Kaneko K., 2012).

Evidenced that THP can inhibits bonding through a stearic barrier rather than crystal attachment areas; binding is not a necessary element of blocking. The manifestations of the discomfort accompanied along with THP either as neither promoter nor inhibitor of crystals which are present in same controversy in urine excretion (Basavaraj DR., 2007).

Nephrocalcin

It is existed in four isoforms namely NC-A, NCB, NC-C, and NC-D. Mustafi and Nakagawa described how the NC can inhibit COM crystal growth and identified binding site for Ca2 + along with procalcin (Mustafi D., 1996). It was finding that the high levels of NC-C and NC-D isoforms were more than NC-A and NC-B in the organic matrix of CaOx crystals in kidney. Isoforms A and B have changed their alignment with Ca2 + binding, no change was observed in the NC-C and NC-D alignment (Nakagawa Y., 1997).

Urinary Prothrombin fragment 1 (UPTF1)

It was the major protein in CaOx matrix of crystals that present in fresh urine. Te major role played by UPTF1 might be due to its characteristics and role in CaOx crystal stone formation (Kleinman J.G., 2004). It was not observed in the struvite crystals, indicating that their discovery in CaOx crystals is the result of direct exposure to crystal composition and not formed as secondary product tissue damage. It has all the expected the potency in undiluted urine as it is a macromolecular urine inhibitor in fresh urine (Ryall RL., 2000). However, it thought to accomplish a prominent role in urinary stone formation, the actual role of UPTF1 should confuse until establishment of relation of this protein and to pathogenesis of stone formation.

Osteopontin

Osteopontin is composed with amino acids such as serine, aspartic acid and glutamic acid. The composed proteins are generally involved in bone formation and biomineralization. It contains negatively charged aspartic acid with high involvement in regulating the body's immune system and disease. The phosphorylated form of OPN only involved in distribution of to broad tissues obtained in combination with calcification of organic matrix of the kidney. The synthesis of OPN was done at kidney and excreted and presented in urine (Utsunomiya M., 1993). Proteins are still widely distributed in the light areas of pancreas, bladder, reproductive tract, intestinal tract, lungs, and chest epithelial cells. OPN was found specifically in the cytoplasm of multiple epithelial cells of distal tubules and collecting ducts (Iline-Vul T., 2020).

Calgranulin

It belongs to the group of S100 protein family calcium binding protein with 28kDa and existed in 3 monomers (A, B and C). It was found to be identified in human urine, kidney and in matrices of various stones such as calcium oxalate (Canales BK., 2010), uric acid stones, and contagious stones or struvite, and in CaP deposits formed by MDCK cells. Its binding capacity to the crystal surface is directly proportional to the inhibitory property of Calgranulin (Pillay SN., 1998).

Renal Lithostathine

It is a glycoprotein present in pancreatic acinar cells extracted from pancreatic juice. The pancreatic juice is a rich source of both calcium and biocarbonates, but their crystal formation can be abolished with lithostathine. Renal lithostathine appears to regulate the precipitation of calcium carbonate crystals. Many studies indicating that early stages of lithiasis are reported with the presence f renal calcium carbonate crystals. These crystals are the initiators for the further promotion of CaOx crystallization and finally supersaturation of urine and supported suitable substrates for different nucleation (Grover PK., 2002).

Human Urinary Trefoil Factor 1 (THF1)

THF1 belongs to the family protein gene. It is composed of mucosal epithelial cells and is expressed in the gastric mucosa. It have the ability to destroy the crystal through inhibition of growth of CaOx into dihydrate form (Thongboonkerd V., 2008). Urine THF is significantly reduced after severe kidney toxicity, and it has already been proposed as a urine biomarker for

Hyaluronic acid

It is present in interstitium of renal mdullary extracellular matrix and also at pericellular matrix mitogen / compressed cells that work in the tubular cells. Size, incorrect ionic state, ideal property to form gel like matrice to make HA a molecule that binds crystal (Verhulst A., 2003).

The secretion from the renal tubules with crystals and formation of Randall's plaque at renal interstitum are also due to binding capacity of HA. In renal cell damage, HA can acts as a promoter for crystal adhesion on the surface of the cell, which will eventually leads to stone formation (Chutipongtanate S., 2005). It is a major crystallization inhibitor which effectively inhibits papillary calcifications, due to the fact that internal HA is low during anti-diuresis; a high risk of crystal formation is most likely during water depletion. On the other hand, too much fluid leads to internal HA that acts against crystal formation.

Annexins

They contain COOH-terminal protein "core" that regulates their membranes and calcium binding properties. The process of attachment of COM crystals to the renal cells are mediated through Annexins and also in their consequent internal formation. The calculi and tubular cell interaction is meditated through subsequent cellular events which finally lead to the renal stones (Kumar V., 2003).

Matrix Gla Protein (MGP)

It is a protein with 84 amino acids is 5 γ-carboxyglutamic acid (Gla) residues with a calcium and phosphate ions and associated with hydroxyapatite crystals. The structure of renal stones is same as seen in vascular calcification: building calcific plaques, increasing the expression of calcification inhibitors, and controlling the active calcification process. MGP genetic single nucleotide

polymorphism was associated with an individual's tendency to nephrolithiasis (Gao B., 2007).

Sialic acid

Sialic acid in urine contains two different types of sialic acid, namely, free and bound sialic acid which is found as a basic component of urine macromolecules. Removal of sialic acid residues from glycosylated proteins proposed as stone inhibitors with inhibitory activity. Desialylated THP has separated from the stone matrix and urine of the stone formers (Webber D., 2006). Glycoproteins containing sialic acid containing, and possibly glycolipids (sialoglycoconjugates), are seen as critical markers of nucleation of COD crystals in a portion of the apical renal cell. Sialic acid containing glycoproteins and / or glycolipids arises from mediating nucleation and growth of COD crystals in the area above each kidney epithelium. The abundance of sialic acid and / or a three-dimensional organization, for example due to genetic mutations or acquired cells adjacent to the proton, can alter crystal nucleation and bind to *vivo*. Excessive exposure to sialyltransferase in the collection of cells of certain stone-producing pathways, perhaps mediated by mutation, may release increased amounts of sialic acid with some carbohydrate interaction in the apical plasma membrane.

Enhanced linkage of sialic acids with galactose residues on glycoprotein cell surfaces may promote the attachment of crystals on tubular layers and grow upon the plasma membrane. In this way, over expression of tubular cell surface sialic acid residues could mediate crystal retention in the kidney and subsequent stone formation in certain predisposed individuals. Presumably, changes in apical membrane glycoproteins of distal rather than proximal tubular cells would be critical because tubular fluid becomes progressively supersaturated along the distal tubule and collecting duct, thereby allowing calcium oxalate crystal nucleation to occur. However, additional studies will be needed to characterize HA crystal nucleation and growth upon control and enzyme-modified renal cells (Verkoelen CF., 2000).

MCP-1monocyte chemoattractant protein-1

MCP-1 is an important component of pathological abnormalities associated with renal epithelial cells, including nephrolithiasis. The expression of MCP-1 in rare cases associated with non-infectious kidney stones and need to study about calcium phosphate and urinary crystals also stimulate renal epithelial cells to produce MCP-1 or not (Segerer S., 2001). MCP-1 levels are elevated in the urine of patients with multiple glomerulonephropathies and are associated with glomerular damage and monocyte infiltration. Patients with active lupus and nephritis show higher levels of urination of MCP-1 than controls or patients with no disease. MCP-1, which is often associated with localized inflammation, may be one of the mediators of chemokine associated with the incorporation of various kidney urine crystals during kidney stone formation (Tohru U., 2003).

Exposure of renal epithelial cells to crystals leads to an increase in OPN, bikunin, heparin sulphate, MCP-1 and prostaglandin E2, which are known to participate in inflammatory processes and the production of an external matrix. Addition of calcium oxalate crystal on rats also activates the renin-angiotensin system. It has been found that high therapeutic potential of anti-oxidants and free radicals providers reduce the recurrence of stones, especially in the aftershock wave of lithotripsy, which is known to produce ROS and cause kidney damage (Khan SR., 2005). Calcium oxalate crystals attach to epithelial cells, while NADPH oxidase forms superoxide, which activates cyclophilin D in mitochondria. The acceleration of MPT has led to mitochondrial collapse, OS, activation of the apoptotic pathway, and expression of OPN in the initial process of renal calcium crystallization (Niimi K., 2014).

Conflict of interests:

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