Pathogenesis of renal calculi

Renal taş hastalığı patogenezi

Ehud Gnessin¹, James E. Lingeman¹, Andrew P. Evan²

¹Methodist Hospital Institute for Kidney Stone Disease, Indianapolis, IN, USA
²Indiana University School of Medicine, Department of Anatomy and Cell Biology, Indianapolis, IN, USA

Abstract

Stone formation is a complex process, mainly because stone disease is a polygenic, multifactorial disorder that involves an interrelationship between the kidney, bone, and intestine. Although great progress has been made in recent years to delineate the exact processes that lead to the formation of renal calculi, there are many incomplete-ly answered questions regarding pathogenesis of stone formation. There are distinct stone phenotypes and the cascade of events leading to kidney stone formation varies depending on this phenotype. Different mechanisms of stone formation have been described for numerous stone types and clinical situations. Herein, we reviewed the current knowledge about the basic pathophysiologic theories involved in the formation of different renal calculi. Key words: Pathogenesis; Randall’s plaque; renal calculi; stone disease.

Nephrolithiasis is a common disease in the United States and worldwide. The lifetime risk for kidney stone disease exceeds 7-12% in the general population and the prevalence of upper tract stone disease has been reported to be increasing.¹ Kidney stones cause considerable suffering and have a substantial economic impact. Pearle et al.² reported that the cost of urolithiasis in the United States was estimated to be more than $2 billion in the year 2000, reflecting a 50% increase since 1994. Although it is perceived as an acute illness, stone disease is now gradually accepted as a chronic systemic disease that may lead to renal loss.³ Stone prevention is important due to recurrence rates without medical treatment of more than 50% over 10 years.⁴⁻⁵ Further knowledge of the underlying mechanism of stone formation can potentially lead to novel therapies targeting the formation process, enhancing prevention efforts.

Pathophysiologic mechanisms of stones are complex, mainly because stone disease is a polygenic, multifactorial disorder that involves an interrelationship between the kidney, bone, and intestine. Much effort has been undertaken in recent years to delineate the pathophysiologic process that leads to the formation of renal calculi. There are distinct stone phenotypes and the cascade of events leading to kidney stone formation varies depending on this phenotype. Different mechanisms of stone formation have been described for numerous stone types and clinical situations.⁶⁻¹¹ The purpose of this report is to review the current knowledge about the basic pathophysiologic theories involved in the formation of different renal calculi.
The physical chemistry of stone formation

Supersaturation and metastable zone

Kidney stones result from a complex physical and chemical process, which involves two major opposing forces. One is urinary supersaturation (SS) that provides the driving force for stone formation. The other is urinary inhibitors and other soluble molecules that protect from formation of calculi. SS is the driving force for a phase change from dissolved salt to solid phase. SS means that the concentration of a stone-forming salt exceeds its solubility in a solution (the solubility product). Once this concentration is reached, nuclei of its solid phase can form.\[12\] Exact SS values can be obtained by measuring the ionic concentration of the main urinary solutes in 24-hour urine tests and combining it with urinary pH. These calculated values have been shown to correlate with stone composition, thus emphasizing the importance of SS in the pathogenesis of stone formation.\[13\]

In urine, a medium much more complex than pure solution, SS may rise up to eightfold, depending on the crystal involved, without new solid-phase formation. The concentration product range between the solubility product and new stone formation is termed the “metastable zone”. The upper limit of the metastable zone is termed the “formation product” and it is the solute concentration at which spontaneous calculi formation may occur. Existing stones may aggregate and grow in the metastable zone but new stone cannot form without a nidus.\[14\]

Nucleation and crystal formation

Nucleation is the establishment of the smallest unit of crystal formation. There are two forms of nucleation: homogeneous nucleation and heterogeneous nucleation. In a pure solution, nuclei will form when SS rises above the formation product. This nucleation process is called homogeneous nucleation and usually requires high SS levels. In human urine the chemical environment is diverse and crystal nuclei tend to form on structures such as cellular debris, urinary crystals, urinary casts, and existing urinary membranes. This form of nucleation is called heterogeneous nucleation and occurs in a much lower level of SS. In fact, most renal calculi contain a mixture of more than one crystal type suggesting that a process of heterogeneous nucleation is responsible for the formation of most stones.\[15\]

Crystal aggregation and epitaxy

Stone crystals bind to one another through a process known as aggregation or agglomeration. Strong chemical and electrical forces promote the aggregation process. Once crystals adhere to one another, they are held in place and cannot be easily separated. Crystal aggregation is thought to have an important role in stone formation since a single crystal would never be large enough to be retained in the collecting system.\[16\]

Most crystals have a lattice structure containing more than one stone type. The process which stones form as a multicomponent crystal include adherence of one crystal on another, formation of a second layer, and overgrowth as a crystal lattice. The ability of one crystalline lattice to grow on another is called epitaxy, and together with crystal aggregation, is thought to play an important role in the formation of urinary calculi.

Crystal retention

Within the time frame of transit of urine through the nephron, crystals must grow or aggregate in order to form urinary calculi. Since the transit time from collecting duct to bladder is estimated to be around 5 to 7 min, crystal retention is necessary for stone formation of a clinically significant size. There have been two mechanisms proposed to account for crystal retention: the free particle hypothesis and the fixed particle hypothesis.\[17\] The free particle hypothesis suggests that the process of nucleation occurs entirely in the tubular lumen. As crystals move through the renal tubules, rapid aggregation generates a crystal large enough to occlude the tubular lumen and to be retained at the level of the papillary collecting duct. This hypothesis has been questioned as the flow of ultrafiltrate was measured to be so rapid as to prohibit crystal aggregation, thus preventing crystals large enough to occlude the tubular lumen.\[18\] The fixed particle hypothesis relies on adherence of crystals to a fixed point, such as renal epithelial cells or Randall’s plaque.\[19\] Although normal urothelium is thought to be resistant to crystal adherence, chemical or mechanical urothelial damage may promote crystal binding and aggregation.

Inhibitors of crystal growth

Although urine SS is important in the formation of calcium-oxalate calculi, other urinary factors
may be equally important. Frequently, urine from non-stone-formers is supersaturated with respect to calcium-oxalate, yet no stone forms in these patients. A number of substances found in the urine have been shown to inhibit formation of calculi. For example, repletion of the urine with citrate will reduce the likelihood of calcium oxalate stone formation through several mechanisms. First, citrate complexes with calcium reduce the availability of ionic calcium to interact with oxalate or phosphate. Second, it directly inhibits the spontaneous precipitation of calcium oxalate and third, it prevents the agglomeration of calcium oxalate crystals. Other modulating molecules include magnesium, pyrophosphate, nephrocalcin, Tamm-Horsfall protein, crystal matrix protein, uropontin, prothrombin fragment one, lithostatine, RNA and DNA fragments, albumin, glycosaminoglycans, and calgranulin.[12]

Role of matrix in the pathogenesis of urinary calculi

All kidney stones contain organic matrix, generally comprising 2-3% of the dry weight of the stone (excluding the rare matrix stone which is usually associated with chronic urinary tract infection).[20] Although the role of organic, non-crystalline matrix in the pathogenesis of renal calculi has not been definitively characterized, it is likely that matrix plays a significant role in the process of stone formation. Scanning electron microscopic studies of calcium oxalate stones have revealed organic material between adjacent crystals, supporting the hypothesis that matrix acts as a ground substance or as a crystal binder.[21] The exact role of matrix in stone formation has yet to be discovered.

Theories of stone pathogenesis

Crystal-induced renal injury

Crystal retention is an essential element of kidney stone formation. One proposed mechanism that would allow retention is tubular injury. Damage to inner medullary collecting ducts or to tubular epithelium may serve as nidus to crystal adherence, thus allowing aggregation and stone formation. In certain animal and tissue culture models, it was possible to induce cellular injury that serves as an anchoring site for crystal attachment.[22] Generally, this has been done by administration of oxalate directly or by providing a precursor of oxalate. Hyperoxaluria results in increased production of reactive oxygen radicals, thus inducing lipid peroxidation and cellular injury.[23] Although there are internal mechanisms that provide protection to reactive oxygen species, in these models of hyperoxaluria-induced damage, the level of oxalate is so high that it will overcome these protective systems. When crystal adherence does occur, it induces renal interstitial inflammation with migration of macrophages. Then interstitial tumor necrosis factor alpha increases which results in elevated levels of metalloproteinases. These, in turn, may induce the erosion of subepithelial crystal deposits which can create a nidus for stone formation.[24]

These models have a number of shortcomings. In most of these studies, the levels of the induced oxaluria are supraphysiologic. Moreover, Holmes et al.[25] have reported that at a physiologic dose of dietary oxalate, no evident renal damage or oxidative stress had occurred. No study has yet demonstrated crystal attachment to a healthy inner medullary collecting duct cell lining since most models are tissue-culture based.

Shock wave induced renal injury

It has been proposed that extracorporeal shock wave treatment (SWL) may be associated with renal injury, which may induce new calculi deposition. Indeed, studies have demonstrated higher stone recurrence rate after SWL than after other treatments.[26-27] A recent study that used a rat model has found that SWL treatment resulted in tubular injury in a dose dependent manner. In turn, tubular injury was associated with a markedly increased deposition of calcium crystals.[28] Moreover, SWL treatment has been associated with conversion from calcium oxalate stones to calcium phosphate stones. Patients who transformed had significantly more SWL treatments than patients who did not.[29] Collecting duct epithelial cells that are responsible for controlling urinary pH may be injured during SWL treatment and may be responsible for the conversion.[30]

Free particle theory

It has been proposed that crystal formation may occur by rapid growth within the papillary collecting ducts. This free particle hypothesis has been debated. Finlayson et al.[18] have stated that crystal mass large enough to occlude the papillary collecting duct can not be created given the rapid transit time through the tubule. Robertson has used mathematical modeling analysis to give supporting explanation to this theory.[31] He suggested that several hydrodynamic factors may assist crystal aggregation and formation and may allow the rapid formation of stone
within the tubules, among which are: fluid drag along tubular walls, its counter effect by the epithelial lining, and the effect of gravity on particles traveling in an upward-draining collecting duct.

**Urine stasis**

Urine retention has been proposed as an etiologic factor for stone formation, although direct evidence is lacking. Several clinical situations may contribute to upper tract urinary stasis such as ureteropelvic junction obstruction, calyceal diverticulum, medullary sponge kidney, hydronephrosis, and horseshoe kidney.[32-33] The impaired drainage of urine from the upper collecting system may allow for crystal retention and eventual calculi formation. Matlaga et al.[33] have demonstrated that urinary risk profiles of patients with calyceal diverticulum calculi were the same as the risk in calcium oxalate stone formers. However, these authors also found that the SS of urine aspirated directly from the diverticular cavity was significantly lower than of the urine found in the renal pelvis, suggesting a role for urinary stasis in the pathogenesis of diverticular calculi. In contrast, Auge et al.[34] have found multiple metabolic abnormalities in 12 out of 12 patients with symptomatic diverticuli disease suggesting that these abnormalities may be the major contributory factor for stone formation. It may be that a combination of both metabolic abnormalities and urinary stasis influence stone formation in calyceal diverticuli.

**Nanobacteria**

Nanobacteria are cytotoxic, gram negative, atypical bacteria detected in bovine and human blood that has been implicated in a variety of disease states such as atherosclerotic heart disease and periodontal disease. Nanobacteria are known to produce carbonate apatite on their cell walls.[35] In vitro animal studies have revealed calcific stone formation by nanobacteria, as well as stone formation following nanobacteria inoculation of rat kidneys.[36] An in vitro study on human kidney stones has demonstrated the presence of nanobacteria on their surfaces.[37] Despite these supportive basic science reports, the role of nanobacteria in stone pathogenesis is controversial and clinical evidence in humans is lacking.

**Randall’s plaque**

As discussed previously, several mechanisms have been proposed for the formation of calcium stones. The free particle and the fixed particle theories included obstruction of tubular lumen either by supersaturation and homogeneous nucleation of stone-forming salts inside the tubular lumen or by crystal adherence to luminal renal tubular cells and interaction of the fixed nuclei with the surrounding supersaturated environment. Both processes occurred within tubular lumen.[38]

Dr. Alexander Randall, more than six decades ago, was the first to argue that intraluminal plugging (termed type 2 attachment by Randall) is an infrequent occurrence in kidney stone formers. He conducted detailed examination of the papillae of more than 1,000 non-selected cadaveric renal units. He observed calcium salt deposits in the tip of the papilla in 19.6% of individuals studied. These plaques were interstitial in location and composed of calcium phosphate crystals.[39] He suggested that interstitial calcium phosphate deposits are initial niduses that anchor urinary crystals to areas of exposed interstitial plaque (Randall’s plaque). Normally, the urothelium would cover the sites of Randall’s plaque and prevent attachment of urinary crystals. However, if erosion of the overlying uroepithelium occurs, sites of interstitial plaque would be exposed to the supersaturated urine that then propagates calcium oxalate crystal deposition. This process results in a developing stone attached to a site of interstitial plaque termed type 1 attachment. Randall’s hypothesis was disputed since it was carried out in cadaveric kidney specimens and not in a targeted kidney stone-forming population. Randall tried to expand his hypothesis to fit all stone formers and the great risk of global theory is that it only requires one exception to disprove the rule. We now know that there are different distinct groups of stone formers and stone pathogenesis is particular to a different clinical setting.

**Randall’s plaque and the pathogenesis of idiopathic calcium oxalate stone formers**

Idiopathic calcium oxalate stone formers, defined as those patients in whom calcium oxalate stones form without any systemic cause other than idiopathic familial hypercalciuria, are the most common type of stone former.[40] When examined with high resolution digital endoscopic imaging, the renal papilla in all such patients were noted to have sites of plaques which were manifested as whitish calcifications located at the papillary tip just as Randall initially described (Fig. 1).[8] Using modern digital endoscopic techniques, Randall’s plaques were visualized
in vivo and renal papillary endoscopic mapping and biopsies were made possible.[41] A study using nephroscopic papillary mapping in 13 calcium oxalate kidney stone formers determined the percent of plaque coverage to be directly correlated with the number of kidney stones formed.[42] Histological examination of the papillary tissue demonstrated that plaque was composed of calcium salts as defined by Yasue metal substitution technique (Fig. 2).[8] Furthermore, the plaque originated in the basement membranes of the thin loops of Henle and these deposits were localized to the inner medullary interstitial space and followed the thin loop of Henle to the basal urothelium. The mineral composition of the interstitial deposits was identified as hydroxyapatite based on Fourier-transform infrared microspectroscopy and electron diffraction analysis. Calcium oxalate crystals were not detected in the tissue or tubular lumens in any of the specimens biopsied. Of note, the investigators also demonstrated that the presence of interstitial hydroxyapatite deposits resulted in no evidence of cellular injury or inflammation.

To test the hypothesis that idiopathic calcium oxalate stones grow on Randall’s plaque deposits, Miller et al.[43] imaged endoscopically nine patients with idiopathic calcium oxalate stones (Fig. 3). All stones were removed intact and recorded by the operating surgeon as being attached or unattached; for all attached stones the surgeon determined if the site of attachment was the plaque. The nine patients had a total of 115 stones; of these, 75% were attached to Randall’s plaque. Further analysis of the unattached stones using micro-computed tomography showed at least one internal region of calcium phosphate within each of these calcium oxalate stones, supporting the hypothesis that in idiopathic hypercalciuria patients, both attached and unattached stones occur as a result of a common pathogenic mechanism; that is, all originate initially as attached to interstitial plaque on the renal papilla.[44]

Clinical studies have suggested a correlation between urine volume, urinary calcium, and severity of stone disease with the fraction of papillary interstitium covered by Randall’s plaque.[42,45-46] It has been proposed that plaque formation in the thin descending loop of Henle occurs because of an increase in interstitial calcium and phosphate concentration, as well as an increase in renal papillary osmolality as a result of water reabsorption in this nephron segment, but clinical evidence for this hypothesis is lacking.
Whether increasing the interstitial pH will result in further plaque formation is still unknown. Further study in this area will elucidate the underlying process of idiopathic calcium oxalate stone formers.

**Stone formation following intestinal bypass**

Patients who undergo jejunal-ileal bypass surgery for morbid obesity have been reported to form calcium oxalate kidney stones and typically have marked hyperoxaluria.\(^{19,47}\) Evan et al.\(^{8}\) studied a cohort of patients who have previously undergone jejunal-ileal bypass procedures and subsequently formed kidney stones. Using digital endoscopy followed by biopsy and histological analysis, these patients were found to harbor no interstitial plaque. However, crystal aggregates were found in the inner medullary collecting ducts. Histologic examination of the papillary biopsies revealed Yasue positive deposits only in the lumen of the inner medullary collecting ducts as far down as the terminal collecting ducts. Electron microscopy found crystals attached to the apical surfaces of the collecting duct cells and in some, completely obstructing the tubular lumen. Moreover, in contrast to conditions in idiopathic calcium oxalate stone formers, there is evidence of renal inner medullary duct cellular injury, interstitial fibrosis, and inflammation adjacent to the crystal aggregates. Crystal deposits in these biopsies from patients with intestinal bypass were hydroxyapatite. This occurred despite overt hyperoxaluria and an acidic urinary environment, implying that tubular pH where deposits occurred may be different from the final urinary pH.

**Patients with ileostomy**

Patients with ileostomy typically have recurrent renal stones and produce low-volume, acidic, sodium-poor urine because of abnormally large enteric losses of water and sodium bicarbonate. Evan et al.\(^{48}\) used intra-operative digital photography and biopsies to determine the morphological changes associated with ileostomy patients. They observed interstitial plaque as predicted from the generally acidic, low-volume urine in these patients. However, all patients had crystal deposits that plugged the Bellini ducts and inner medullary collecting ducts. Despite acidic urine, all crystal deposits contained apatite and five of seven patients had deposits of sodium and ammonium acid urate. Stones were either uric acid or calcium oxalate as predicted by supersaturation; however, there was a general lack of supersaturation for calcium phosphate, sodium, or ammonium acid urate because of the overall low urine pH. Despite low urine pH, patients with an ileostomy resembled those following bypass surgery in which inner medullary collecting duct apatite crystal plugs are found. They are, however, unlike these bypass patients having interstitial apatite plaque, presumably due to low urine volume.

**Brushite stone formers**

Calcium phosphate stone formers comprise about 15% of the stone forming population and the incidence of calcium phosphate stones may be increasing.\(^{29}\) Brushite (calcium monohydrogen phosphate) stones comprise about a quarter of calcium phosphate stones. In brushite stone formers, similar to calcium oxalate stone formers, there is evidence of cell injury and interstitial fibrosis in the inner medullary collecting ducts adjacent to apatite crystal deposits following gastric bypass surgery.\(^{10}\) Interstitial changes were also detected in cortical biopsies along with advanced glomerulosclerosis, tubular atrophy, and interstitial fibrosis. The endoscopic examination of renal papillae in brushite stone formers has consistently demonstrated three types of deposits. The first pattern was sites of Randall’s plaques, as seen in idiopathic calcium oxalate stone formers (Fig. 4). The second pattern was large, yellow deposits projecting from the opening of ducts of Bellini into the urinary collecting space (Fig. 5). The third was suburothelial yellow deposits on the sides of the papillary tips and clearly within the lumen of inner medullary collecting ducts (Fig. 6A, B).

**Cystine stone formers**

Cystinuria is an autosomal recessive genetic disorder characterized by a defect in dibasic amino acid transporters. Evan et al.\(^{6}\) have reported on the gross and microscopic pathology of the renal papilla, medulla, and cortex of cystine stone formers. Endoscopically ducts of Bellini were dilated and were plugged with cystine crystals. Crystal plugs often projected into the urinary space. Histological examination revealed cystine crystallization in ducts of Bellini with cell injury, interstitial reaction, nephron obstruction, and the potential of inducing cortical change and loss of inner medullary collecting duct tubular fluid pH regulation resulting in apatite formation. Indeed, abundant apatite crystals were identified in the lumens of loops of Henle. The patterns
of inner medullary collecting duct dilation and loss of medullary structures is most compatible with obstruction, either from Bellini duct lumen plugs or urinary tract obstruction from stones.

Distal renal tubular acidosis

Distal renal tubular acidosis (dRTA) is a metabolic disorder characterized by a non-anion gap metabolic acidosis, accompanied by an alkaline urinary pH that does not fall appropriately during an exogenous acid load. Hypokalemia is frequently present due to renal potassium losses. Patients with dRTA often present a radiographic picture of nephrocalcinosis and typically harbor calcium phosphate stones in the form of hydroxyapatite (Fig. 7A, B). Evan et al.[49] reported five stone forming patients with dRTA. The authors used intra-operative digital endoscopy for papillary and cortical biopsies. The main abnormalities observed were plugging of inner medullary collecting ducts and ducts of Bellini with deposits of calcium phosphate in the form of apatite. Plugged ducts were surrounded by interstitial fibrosis, but the fibrosis was generalized and was a main feature of the histopathology even when plugging was not present. Epithelial cell injury was abundant. Compared with brushite stone formers and patients with cystinuria, ductal plugging was more pronounced and the fibrosis was widespread.
Conclusion

Stone formation is a complex process (Fig. 8) involving not a single pathway but several different processes, dependent on the appropriate clinical scenario. The field of stone pathogenesis is still replete with many incompletely answered questions; however, great progress has been made in recent years to delineate the exact processes that lead to the formation of renal calculi. Meticulous renal mapping, papillary and cortical biopsies, and subsequent detailed analysis have not only provided powerful evidence for the dominant role of Randall’s plaque in the pathogenesis of calcium oxalate renal calculi but have also demonstrated that the histology of the renal papilla is particular to the clinical setting. Further studies aimed at gathering metabolic information, such as measuring intraductal urinary parameters in vivo, will aid us to develop better strategies for understanding stone formation and for the diagnosis and treatment of stone-forming patients.
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Conflict of interest

No conflict of interest was declared by the authors.

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Correspondence (Yazışma): Dr. James E. Lingeman.
1801 North Senate Blvd., Suite 220, 46202 Indianapolis, United States.
Phone: +1 317 962 24 85 e-mail: jlingeman@clarian.org