Published in final edited form as:

J Urol. 2018 January ; 199(1): 186–192. doi:10.1016/j.juro.2017.08.063.

Papillary Ductal Plugging is a Mechanism for Early Stone Retention in Brushite Stone Disease

James C. Williams Jr.^{*}, Michael S. Borofsky, Sharon B. Bledsoe, Andrew P. Evan, Fredric L. Coe, Elaine M. Worcester, and James E. Lingeman[†]

Departments of Anatomy and Cell Biology (JCW, SBB, APE) and Urology (JEL), Indiana University School of Medicine, Indianapolis, Indiana, Department of Urology, University of Minnesota (MSB), Minneapolis, Minnesota, and Section of Nephrology, University of Chicago (FLC, EMW), Chicago, Illinois

Abstract

Purpose—Mechanisms of early stone retention in the kidney are under studied and poorly understood. To date attachment via Randall's plaque is the only widely accepted theory in this regard, which is best described in idiopathic calcium oxalate stone formers. Brushite stone formers are known to have distinct papillary morphology relative to calcium oxalate stone formers. As such we sought to determine whether stone attachment mechanisms in such patients may be similarly unique.

Materials and Methods—Patients undergoing percutaneous and or ureteroscopic procedures for stone removal consented to endoscopic renal papillary examination and individual stone collection. Each removed stone was processed using micro computerized tomography to assess the 3-dimensional microstructure and the minerals contained, and search for common structural features indicative of novel mechanisms of early growth and attachment to renal tissue.

Results—A total of 25 intact brushite stones were removed from 8 patients and analyzed. Video confirmed attachment of 13 of the 25 stones with the remainder believed to have been accidentally dislodged during the procedure. Microscopic examination by light and computerized tomography failed to show evidence of Randall's plaque associated with any stone containing brushite. Conversely each brushite stone demonstrated microstructural evidence of having grown attached to a ductal plug formed of apatite.

Conclusions—Three-dimensional analysis of small brushite stones suggests overgrowth on ductal apatite plugs as a mechanism of early stone growth and retention. Such findings represent what is to our knowledge the initial supporting evidence for a novel mechanism of stone formation which has previously been hypothesized but never verified.

^{*}Correspondence: Department of Anatomy and Cell Biology, School of Medicine, Indiana University, 635 Barnhill Dr., MS5055Y, Indianapolis, Indiana 46202 (telephone: 317-278-7494; FAX: 317-278-2040; jwillia3@iupui.edu).

[†]Financial interest and/or other relationship with Beck Analytical Services.

The corresponding author certifies that, when applicable, a statement(s) has been included in the manuscript documenting institutional review board, ethics committee or ethical review board study approval; principles of Helsinki Declaration were followed in lieu of formal ethics committee approval; institutional animal care and use committee approval; all human subjects provided written informed consent with guarantees of confidentiality; IRB approved protocol number; animal approved project number.

Keywords

kidney calculi; calcium phosphate; dibasic; dihydrate; tomography; x-ray computed; etiology; kidney tubules; collecting

Small stones that form in the kidney should be passed by a normal urinary system. Indeed, this is the functional principle of shock wave lithotripsy, in which stones in the kidney are broken into small pieces which then pass harmlessly from the body.¹ This fact represents a sort of mystery concerning the growth of a kidney stone de novo. A stone must begin as a small concrement in the renal urinary space but in a stone former it must be prevented from being passed before it can grow into a clinically significant stone. Thus, the growth of stones in the kidney must involve some mechanism or mechanisms to retain the early stone while it grows to a sufficient size that cannot be passed.

While it is conceivable that stones could grow in the urinary space so quickly that they would be trapped above the ureteropelvic junction, modeling based on urinary supersaturation does not support the possibility of typical stones growing at such a rapid rate.² It is more likely that stones are retained in the kidney by some mechanisms other than size alone during early growth.

One such mechanism of stone retention in the kidney is that of interstitial Randall's plaque. In some CaOx stone formers calculi have been shown to form solely on Randall's plaques.^{3,4} In this kind of stone former the interstitial plaque becomes exposed to calyceal urine and a stone grows quite slowly as CaOx is deposited on the plaque layer by layer.⁵ The early calculus is retained in the renal calyx by the anchoring of the small stone to the collagenous connective tissue of the renal papilla via the Randall's plaque.⁶ Stones growing in this manner can eventually be freed from the papillary anchor^{7,8} but they retain the mineral signature that shows the early origin on papillary plaque.⁹

However, it also seems that some forms of kidney calculi can be retained in the calyx by beginning as overgrowths on ductal plugs.^{10,11} In this scenario mineral is deposited in the renal tubule and becomes lodged in the lumen of the terminal collecting duct. The distal end of such a ductal plug can then be a site for growth of an early stone as mineral is deposited on the end of the plug from calyceal urine. As long as the plug remains lodged in the collecting duct the nascent stone will be held in the kidney and allowed to grow.

Evidence for this mechanism of early stone retention has been more difficult to collect than it has been for the Randall's plaque mechanism for retaining CaOx stones. In a CaOx stone, it is relatively easy to see by micro CT the remnant of Randall's plaque that shows that the stone originated on the papilla.¹² In other kinds of stones the remnant of an apatite plug can be much more difficult to discern.¹³

Thus, although a number of groups have assumed that stones can grow on ductal plugs¹⁴ and that ductal plugs might be a mechanism for retention in the kidney during early growth,⁵ to our knowledge that hypothesis has never been explored in any direct manner. We studied a series of patients in whom brushite stones formed and make the case that in this kind of

stone former the retention of nascent stones on ductal plugs is most certainly a mechanism of early stone growth.

MATERIALS AND METHODS

Among the same population of patients in which we studied previous stone conditions^{3,10,11} there were 28 who produced stones that contained at least some brushite. These patients were studied for evidence of early mechanisms of stone retention via attachment to the renal papillae.

Briefly patients were recruited for analysis who were undergoing ureteroscopy and/or percutaneous nephrolithotomy and consented to digital endoscopic study of the papillae during surgery as well as detailed collection of stone specimens. Stone specimens were collected as much as possible one by one and on a papilla by papilla basis. A key part of the experimental approach in this study was obtaining intact stones as we reasoned that damage to a stone could easily obscure clues to stone formation. We used micro CT to confirm the intact nature of the stones and identify minerals and their relationships in these small stones. We have previously documented the ability of micro CT to identify minerals in kidney stones.^{15–17}

Each stone specimen was scanned by micro CT¹⁷ using a voxel size of 2 to 14 μm depending on specimen size. Micro CT was used to judge mineral types and mineral identifications were confirmed in every patient by infrared spectroscopy.¹⁸ Apparent ductal plugs on small stones were identified by shape and size using micro CT slices and 3D surface renderings.

Simple comparisons were done using the Wilcoxon signed rank test with significance considered at $p < 0.05$.

RESULTS

In 20 of the 28 patients in whom stones were formed of brushite no small brushite stones were collected. Patients with brushite stones tend to have a large stone burden.¹⁹ It may be that the mass of stone material removed in these patients was so large that any nascent stones, which are difficult to collect under any circumstance, were overlooked or simply dislodged by fragments of larger stones. Indeed, in the 20 patients with no brushite stones that appeared to have been attached to papillae the average $\pm$ SD total volume of stone removed and scanned by micro CT was $1.7 \pm 2.2 \text{ cm}^3$. However, in the 8 patients in whom at least 1 stone was collected that had apparently been attached to a papilla the mean total volume of stone removed was only $0.5 \pm 0.9 \text{ cm}^3$. Thus, in these 8 patients the amount of stone material may have been low enough to allow the visualization and collection of nascent calculi.

The 8 patients included 4 men and 4 women at an average age of 45 ± 9 years, of whom only 1 reported a family history of stones. All 8 patients had undergone bilateral stone removal. The average number of collection units (stone groups, fragment sets or individual stones) was 10.1 ± 6.9 , showing the care with which specimens were collected in these

patients. Serum and urine analyses in the 8 patients were unremarkable for calcium stone formation except urine was relatively alkaline (mean 6.19 ± 0.28) in a range previously reported.^{11,19}

Figure 1 shows representative stones from 6 patients in whom stones were removed from the tip of the renal papillae and collected immediately. Thus, these stones were documented as being attached to the papillae and, despite the obvious diversity of structure in many respects, all of them showed growth on what appeared to be ductal plugs of mineral. On 15 of the 16 calculi from these 6 patients the ductal plugs were composed of apatite. The apatite making up the ductal plugs was sometimes highly layered as in patient 5 and other times rather solid as in patient 4 (fig. 1). This is much as has been described for the variation in morphology of kidney stones formed from apatite.²⁰ One stone in patient 3 contained apatite and CaOx as part of the apparent ductal plug (fig. 1).

In 2 other patients small stones containing brushite were retrieved that appeared to be intact and also showed apparent ductal plugs (fig. 2). Patient 7 yielded 9 such calculi, of which all showed apparent plugs composed of apatite, but patient 8 yielded only 1. In 7 of the 8 patients (21 of the 25 stones) CaOx was the mineral in contact with the ductal plug with brushite crystals extending from CaOx (figs. 1 and 2). Only in patient 5 were the brushite crystals apparently growing directly on the apatite plug (fig. 1). This pattern was seen in all 4 stones from patient 5.

The average width of the plugs in the 25 stones from the 8 patients was $580 \pm 254 \mu\text{m}$ with a smallest diameter of $214 \mu\text{m}$. These widths are consistent with the plugs having filled the lumens of dilated terminal collecting ducts. The nominal width of a renal papilla is approximately 1 cm and so the size of these plugs is significant. Average plug length was $1,097 \pm 518 \mu\text{m}$ and the average maximum stone dimension was $3.3 \pm 1.6 \text{ mm}$.

Video collected during endoscopy revealed visual evidence of ductal plugging with mineral in all 8 patients. Figure 3 shows papillae from patient 6, which were typical. Figure 1 shows the small calculus from patient 6, which was found attached. Figure 4 shows this nascent stone in more detail. Micro CT of this stone revealed a cylindrical piece of apatite which appears to have been broken and was located on the tissue side of the stone where it was attached. The diameter of this apatite cylinder was $214 \mu\text{m}$, a size consistent with the cylinder having been part of a ductal plug since terminal collecting ducts have a nominal diameter of about $150 \mu\text{m}$. The apparently upper end of the apatite plug away from the break was embedded in CaOx along with more apatite. Brushite crystals were growing at the surface of the CaOx/apatite region.

Figure 5 shows the stone that obstructed the upper ureter in patient 6. This large stone with a maximum dimension of 9.1 mm was composed primarily of brushite but it had an extension on 1 side that was approximately cylindrical and contained apatite. High resolution examination of this region revealed an elongated rod of apatite with a width varying from 210 to $300 \mu\text{m}$ and an overall length of about $1,400 \mu\text{m}$. The distal end of this presumed apatite plug was covered with brushite but the end near the body of the calculus was associated with the only region of CaOx in the stone. This in turn was apparently the site of

growth of the brushite that made up the bulk of the stone. Thus, this stone showed the same pattern as most stones in figures 1 and 2. The initiation of calyceal mineral deposition on the presumed ductal plug was CaOx with brushite growth following.

DISCUSSION

Brushite stone formers tend to have a large stone burden and recurrent stone events.¹⁹ Additionally, papillae in brushite stone formers demonstrate dramatic damage and extensive ductal plugging.²¹ However, the link between this papillary pathology and the clinical events has been only conjectural.

We propose a likely explanation. Ductal plugs formed of apatite, which manifest as gross yellow deposits²² and likely papillary damage via crystal nephropathy,²¹ come into contact with the urinary space and in turn serve as nidi for small stones, which are retained in the kidney and allowed to grow to clinical size. Of the 28 patients with brushite stones we identified 8 with a total of 25 apparently nascent stones. All of these calculi showed micro CT evidence of apatite extensions which likely had been ductal plugs, some demonstrably so, with the ductal plug visible as the stone was removed from the papilla. Of the 25 stones 21 showed CaOx deposition on the apatite, followed by brushite. Average maximum dimension of these nascent stones was 3.3 mm, approaching the size expected to be clinically relevant. An additional stone with internal architecture showing a recently covered ductal plug was the symptomatic stone leading to the patient procedure (fig. 5).

Ductal plugs of mineral have been known to exist for some time²³ but the relevance to clinical stone formation has been difficult to establish. It seems likely that the formation of a mineral plug in a papillary collecting duct would require initial adherence of mineral to the epithelium lining the duct.²⁴ However, there are conditions in which agglomerated crystals might form rapidly enough to become lodged in the terminal collecting duct.²⁵ Ductal plugs are known to exist in a number of different kinds of stone formers²⁶ but only limited evidence of a possible role in early stone growth has been presented.¹¹

Demonstrating a connection between ductal plugs and early stone growth has been made difficult because of size and composition. Ductal plugs are almost always less than 1 mm in diameter and they are usually composed of apatite. This is a common mineral in the stones of almost all kinds of stone formers in whom ductal plugs have been identified.^{26,27} Thus, if a stone forms on a ductal plug and is then released from the papilla and receives mineral overgrowth to hide the plug, identifying the plug in the stone can be quite challenging, especially if stone overgrowth mineral includes additional apatite.¹³

A strength of the current study is the use of micro CT, which enables the exploration of stone structure at high resolution. Moreover, the appearance of apatite by micro CT is unique relative to CaOx or brushite, which made it easy to identify minerals in the current series. However, the spatial resolution of micro CT is inversely proportional to the size of the specimen so that identifying apatite plugs in larger specimens is more difficult. Additionally, larger stones must be broken to be removed, making it more unlikely that the stone nidus would be preserved. Thus, the surgical technique in our study was also essential. Harvesting

tiny calculi is technically challenging. Visualizing them in place and then retrieving them intact is even more difficult.

The main limitation of this series is that we cannot specify the generalizability of these data. That is, although all brushite stones that we examined in this study showed evidence of growth beginning on ductal plugs, we cannot extend such a statement to all stones that grow in this type of patient. Specifically although we never saw brushite on a stone growing on Randall's plaque (at least 1 patient had a CaOx stone growing on Randall's plaque but with no brushite), we cannot rule out such a possibility.

This study also does not provide resolution to important questions of why, for example, apatite forms in certain situations rather than another calcium salt such as brushite or even CaOx. The occurrence of apatite as the mineral in ductal plugs is common even when the bulk of urine is too acidic to allow for apatite precipitation.²⁶ Spatial separation was seen in the current study between the mineral forming in ductal plugs (apatite) and the mineral forming from urine (brushite) but we have no explanation of this phenomenon.

CONCLUSIONS

Ductal plugs composed of apatite are common in patients in whom brushite stones form. Such plugs can form the nidus for early stone growth. If the end of the ductal plug extends into the calyceal urine, brushite can be deposited and such calculi can grow to clinically significant size. In 84% of the stones with ductal plugs, overgrowth from calyceal urine was initially CaOx, followed by brushite but the significance of this mineral pattern is unclear. We conclude that ductal plugs formed of apatite are the likely mechanism by which brushite stones are retained in the kidney during early stone growth.

Acknowledgments

Supported by National Institutes of Health Grant P01 DK056788.

Abbreviations and Acronyms

3D	3-dimensional
CaOx	calcium oxalate
COD	CaOx dihydrate
COM	CaOx monohydrate
CT	computerized tomography

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Figure 1.

Representative nascent stones from brushite stone formers which were visualized as adherent to tip of renal papilla, shown as 3D surface rendering with micro CT image stack cut away to reveal presumed ductal plug. Numbers indicate number of small stones in patient for whom stone shown is representative.

Figure 2.

Nascent stones from brushite stone formers that were found loose in urine during procedure. Stones presumably had been anchored to renal papillae but were dislodged during removal of larger stones in kidney. Stones are shown as 3D surface rendering with micro CT image stack cut away to reveal presumed ductal plug. Numbers indicate number of small stones in patient for whom stone shown is representative.

Figure 3.

Images of 8 papillae mapped in kidney of brushite stone former patient 6. Mineral is apparent as well as deformations of papillary tips. Arrowheads indicate some apparent ductal plugs. Circle in upper pole 1 indicates small stone that is subject of figure 4.

Figure 4.

Small attached stone removed from upper pole 1 (fig. 3). *A*, photograph of stone on mm graph paper. *B*, 3D reconstruction of micro CT of stone cut away to reveal what appears to be stub of apatite plug. When surface rendering was rotated in 3D, apatite region clearly showed irregularly flat end with appearance of broken rod. *C*, micro CT slice of stone in plane similar to that of cutaway (*B*). Apparent ductal plug is indicated with apatite identified by characteristic brightness surrounding dark voids. At distal (upper) end of apatite plug COD crystals grew, which in places showed conversion to COM monohydrate. Small apatite mass at upper right apparently grown from urine shows characteristic brightness with rather

large x-ray lucent interior. Note brushite blooming on surface. Brushite is identified by brighter than COD appearance and thin, radially oriented crystals.

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Figure 5.

Symptomatic stone from same patient as in figures 3 and 4. *A*, stone on mm graph paper. *B*, surface rendering of stone from micro CT. *C*, maximum intensity projection (*MIP*) of micro CT stack reveals brightest voxels through stone. Stone bulk was pure brushite. Dashed oval indicates region of what appeared to be apatite rod that originated as ductal plug. *D*, high resolution (*hi-res*) micro CT slice through plug region, which was dissected off stone for this scan. Apatite rod which may have formed as ductal plug is oriented horizontally. Mineral near the distal urine end of presumed plug is dominated by CaOx. Brushite appeared on COD crystal surface and on apatite of presumed ductal plug. Dashed line indicates plane of section (*E*). *E*, slice across apparent ductal plug. Cross-section of apatite rod reveals brushite crystals radiating out from surface. Gray region close to apatite rod suggests initial growth of CaOx before brushite (figs. 1 and 2).