Urol Res (2010) 38:249–256
DOI 10.1007/s00240-010-0290-5

SYMPOSIUM PAPER

The tubular epithelium in the initiation and course of intratubular nephrocalcinosis

Benjamin A. Vervaet · Anja Verhulst · Marc E. De Broe · Patrick C. D'Haese

Received: 23 June 2010 / Accepted: 24 June 2010 / Published online: 2 August 2010

Abstract Intratubular nephrocalcinosis is defined as the histological observation of calcium oxalate and/or calcium phosphate deposits retained within the lumen of the renal tubules. As the tubular epithelium is the primary interaction partner of crystals formed in the tubular fluid, the role of the epithelial cells in nephrocalcinosis has been investigated intensively. This review summarizes our current understanding on how the tubular epithelium mechanistically appears to be involved both in the initiation and in the course of nephrocalcinosis, with emphasis on in vivo observations.

Keywords Nephrocalcinosis · Tubular epithelium · Dedifferentiation · Crystal clearance

Introduction

Intratubular nephrocalcinosis is defined as the histological observation of calcium oxalate (CaOx) and/or calcium phosphate (CaP) deposits retained within the lumen of the renal tubules. This histopathological feature is particularly associated with a variety of disorders and conditions and can be found in renal transplant patients [1], preterm infants [2], patients with either obesity-related jejuno-ileal resection [3], brushite stones [4] or CaP stones due to hyperparathyroidism [5]. Therefore, intratubular nephrocalcinosis is considered to be a symptom rather than actually being an independent pathology. Like any other bio-mineralization process its initiation and progression is determined by a complex interplay between physicochemistry and cell biology. As intratubular nephrocalcinosis is characterized by the intimate contact of crystals with the tubular epithelial cell layer, the question "What is the role of the tubular epithelium in nephrocalcinosis?" has intrigued researchers for decades. Here, a concise overview of our current understanding of this role both in the initiation and in the course of nephrocalcinosis is presented.

Proceedings paper from the third international urolithiasis research symposium, Indianapolis, Indiana, USA, 3–4 December 2009.

B. A. Vervaet · A. Verhulst · M. E. De Broe · P. C. D'Haese (✉)
Laboratory of Pathophysiology,
University of Antwerp, Campus Drie Eiken,
Universiteitsplein 1, 2610 Antwerp, Belgium
e-mail: Patrick.Dhaese@ua.ac.be

B. A. Vervaet
e-mail: benjamin.vervaet@ua.ac.be

A. Verhulst
e-mail: anja.verhulst@ua.ac.be

M. E. De Broe
e-mail: marc.debroe@ua.ac.be

The tubular epithelium during initiation of nephrocalcinosis

The development of intratubular nephrocalcinosis is determined by two key processes, *crystal formation* and *crystal retention*. Driven by the requirement of body fluid, electrolytes and acid–base homeostasis, the concerted actions of the different tubular epithelial cell types along the functional segments of the nephron drastically modulate ion composition, pH, osmolarity and volume of the intratubular fluid before it is finally excreted. These changing conditions may frequently challenge the solubility of the urinary calcium salts, *calcium oxalate* and *calcium phosphate*, and crystals may form in the tubular fluid as a result of supersaturation [6, 7]. Generally, the risk of calcium salt crystallization along the nephron is highest, where ion and water transport are uncoupled,

i.e. the loop of Henle and the collecting duct [6–9]. Critical determinants of the probability, extent, location and type of crystals formed are the active concentrations of the respective ions and the pH of the tubular fluid [7, 10–14]. Pathological conditions or drug (ab)use affecting proper tubular calcium, phosphate, oxalate and acid–base handling may importantly influence this risk profile and not only significantly increase the propensity of crystal formation in the tubular fluid, but may also move it upstream, even up to the proximal tubules in extreme cases [14–21]. As crystal formation and excretion (crystalluria) is common, but harmless in the healthy population [22, 23], the question can be raised whether an increased level of crystal formation on its own is sufficient to result in crystal retention. Interestingly, there are indications that the tubular epithelium, besides its (patho)physiological contribution to *crystal formation*, is also involved in the development of intratubular nephrocalcinosis on a more profound level, i.e. *crystal retention*.

Ever since Finlayson and Reid mathematically inferred that crystals generally cannot grow large enough during their short (3–4 min) transit time through the renal tubules to be retained by their size (i.e. a "free particle" mechanism), it was concluded that the development of intratubular nephrocalcinosis primarily depends on adhesion of urinary crystals to the tubular epithelium (i.e. a "fixed particle" mechanism) [24] (Fig. 1). It should be noted that for the majority of patients with nephrocalcinosis the precise nephron segments presenting crystal deposition have not been identified. However, as suggested by the (patho)physiological crystallization risk profile and already confirmed by a few histopathological studies in patients with acute phosphate nephropathy or renal stones of different aetiology [4, 5, 18, 25], the tubular epithelia of the distal nephron segments generally are considered the main crystal interaction partners. Although it has now been established that crystal retention by size may occur in pathologies presenting extreme crystal formation and/or aggregation (i.e. acute phosphate nephropathy and primary hyperoxaluria), intratubular crystals (with diameters smaller than that of the tubular lumen) are frequently found adhering to the tubular epithelium, particularly in pathologies presenting moderate to even normal physiological levels of crystal formation as seen in transplant patients and preterm infants [1, 25–32]. These observations, therefore, suggest an important direct role for the tubular epithelium in the initiation of intratubular nephrocalcinosis. The main questions posed in this area of research are: (1) "What is the (molecular) phenotype of the crystal-binding epithelia?" and (2) "If this epithelial phenotype is aberrant from the normal situation, is its presence cause or consequence of crystal adhesion?"

The (molecular) phenotype of crystal-binding epithelia

Histopathological observations in patients with nephrocalcinosis or nephrolithiasis of different aetiology reported an association between intratubular crystal deposits and renal injury [33–38]. More detailed examination of the tubulointerstitial changes in the vicinity of intratubular crystals in animal models of nephrocalcinosis further illustrated this association and demonstrated that crystals can be found in contact with the surface of injured/regenerating epithelial cells, apoptotic and/or necrotic cells and even denuded basement membranes [39–42]. Likewise, in vitro, exposure of primary or immortalized tubular epithelial cells to CaOx crystals, showed that these preferentially adhere to either injured, apoptotic, depolarized, immature, migrating or proliferating tubular epithelial cells and not to fully differentiated epithelia [43–47]. In this context it is important to note that literature data indicate that proximal tubular cells bind crystals regardless of their differentiation status, whereas distal tubular cells, which physiologically are more likely to encounter crystals, only bind crystals when they are dedifferentiated [48]. The capacity *not* to bind crystals thus is acquired by the distal tubular epithelium upon differentiation. Although the specific differences between proximal and distal tubules in this respect have not been investigated, mechanistic insight in crystal adhesion was particularly obtained by identifying the luminal membrane characteristics and molecular composition of the crystal-binding (distal) epithelia and led to the discovery of several crystal-binding molecules.

Crystal-binding molecules, in the context of nephrocalcinosis, are considered cell-surface molecules with affinity for crystals that are expressed or produced by renal tubular epithelial cells and allegedly are capable of anchoring crystals to the luminal membrane of those cells. Importantly, these molecules are not or only scarcely present at the luminal membrane of fully differentiated epithelia, however, are upregulated or redistributed to the apical membrane under conditions of cellular dedifferentiation, i.e. injury and repair [46, 47, 49–52]. Currently, four categories of crystal-binding molecules have been identified in vitro: (1) terminal sialic acid residues [53, 54], (2) phospholipids, i.e. phosphatidylserine [52, 55], (3) membrane bound proteins, i.e. collagen IV [56], osteopontin (OPN) [57–59], annexin 2 (ANX2) [60, 61] and nucleolin-related protein (NRP) [47, 51, 62] and (4) glycosaminoglycans, of which hyaluronan (HA) appears to be the most potent crystal-binding polysaccharide [49]. The variety in the nature of these molecules and the fact that no unique crystal-binding molecule has been identified so far indicates that the molecular composition of the crystal-binding membrane may depend on variations in pathophysiological and/or experimental conditions. It is intriguing, however, that all currently known

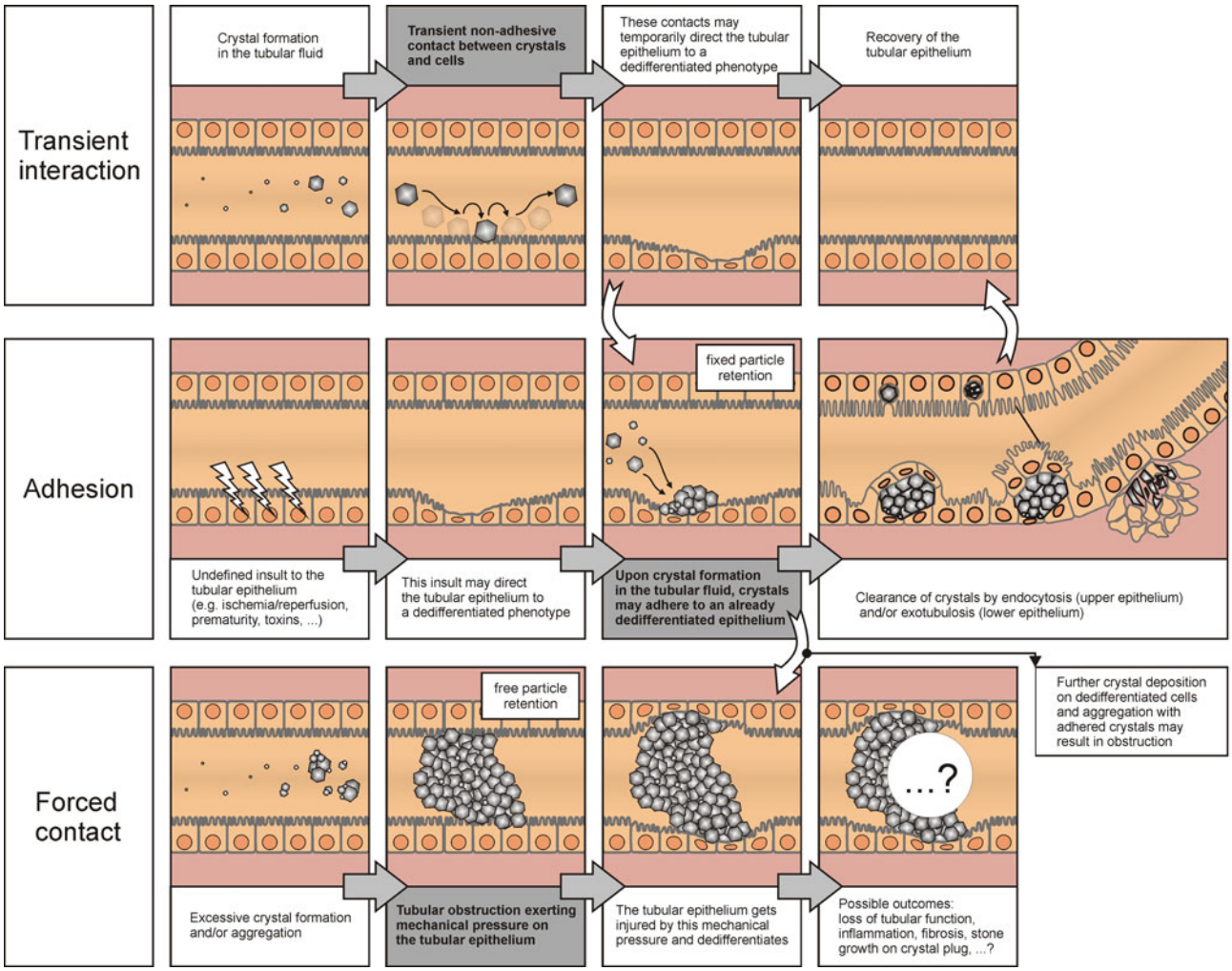

Fig. 1 Types of crystal–cell interactions. The scheme provides an overview of the different types of crystal–cell interactions and possible scenarios which are currently thought to be relevant to the role of the tubular epithelium in the development (and clearance) of intratubular nephrocalcinosis and/or nephrolithiasis. Although this figure has limitations it is an attempt to visually integrate our current understanding of intratubular crystal deposition processes

crystal-binding molecules contribute to a negative cell-surface charge, i.e. a feature which has been shown to be important in crystal adhesion to renal epithelial cells [53, 54, 63]. Therefore, it can be suggested that a spectrum of aberrant phenotypes may bind crystals as long as a sufficient amount and/or properly orientated negative charges are present on the luminal membrane. Investigating the molecular pathways leading to the luminal presentation of these crystal-binding molecules may one day provide potential targets for prevention of crystal adhesion.

The crystal-binding phenotype: cause or consequence of crystal adhesion?

In the literature the question frequently arises whether crystal adhesion either is the consequence or the cause of the phenotypical alterations associated with epithelial injury and repair. The fact that in vitro adhesion of crystals particularly occurs at injured/regenerating epithelia and not at normal cells [43–45] suggests that a dedifferentiated phenotype causes crystal adhesion. However, crystals (and/or concomitant high concentrations of calcium, oxalate or phosphate) have been found to induce injury, proliferation, production of inflammatory mediators and oxidative stress upon contact with epithelial cells in vitro, suggesting that epithelial dedifferentiation is a consequence rather than a cause of crystal adhesion [64–68]. Although these findings appear to contradict each other, they actually challenge our definition of crystal adhesion compelling us to consider that crystal adhesion is not the only type of crystal–cell interaction. In fact, three types of crystal–cell interactions can be envisioned (Fig. 1): (1) adhesion to cell-surface crystal-binding molecules which are expressed by injured/regenerating cells, (2) weak, transient interactions as expected

during crystalluria when crystals passing with the tubular fluid flow shortly get in contact with the tubular wall and (3) a forced crystal–cell contact as expected during obstruction of the tubular lumen. As crystals do not adhere to normal epithelial cells, it is highly unlikely that crystal adhesion is the primary cause of cellular injury and phenotypical alterations. Rather, forced contacts and transient interactions, which may occur to epithelia with a normal phenotype, are to be considered as crystal–cell interactions with initial injury inducing potential. Although the exact mechanical and/or chemotoxic nature of crystal-induced epithelial injury remains to be determined, non-adhesive interactions may direct the normal epithelium towards a crystal-binding phenotype in such a way that a crystal may induce its own adhesion or that of a subsequently passing crystal (Fig. 1).

Thus, with respect to the "cause versus consequence" issue, it can be inferred that crystal adhesion is a consequence and not the initial cause of epithelial injury in vitro. However, progress in our understanding of the pathophysiological mechanisms underlying intratubular nephrocalcinosis necessitates verification of these results in animals and humans. Therefore, as the answer to the question "Is crystal adhesion a consequence of an altered epithelial phenotype?" has been evidenced in vitro, the next question should be "Can evidence also be provided for this mechanism in vivo?".

A *first* indication hereto is provided by experiments investigating the effect of deliberately induced epithelial injury on crystal retention. Kumar et al. [69] observed that hyperoxaluric rats developed markedly more crystal deposition upon induction of renal injury by administration of gentamycin. Likewise, in a more relevant clinical setup, Xue et al. [70] demonstrated that shock wave induced renal injury resulted in more crystal retention in the injured kidney than in the contralateral unshocked kidney upon administration of ethylene glycol (EG).

A *second* line of indirect support comes from studies aiming at diminishing or abolishing crystal deposition by treating the animals with compounds that tend to prevent renal injury. As production of reactive oxygen species has been identified as an important aspect of renal injury [71–75], prevention particularly focussed on restoring the normal oxidation status of the renal tissue. For example, treatment with Vitamin E, a fat-soluble anti-oxidant, prevents EG-induced CaOx crystal deposition in the kidney by attenuation of tubular injury [76, 77]. In analogy, the cholesterol lowering drug Atorvastatin, which has anti-inflammatory and anti-oxidant activities, reduces both tubular injury and crystal deposition in the EG model [78]. Likewise, Ito et al. [79] reported that the anti-oxidative effect of green tea decreased renal CaOx crystal retention in rat kidneys challenged with EG and vitamin D. Furthermore, reduction of angiotensin II production via inhibition of angiotensin converting enzyme or blocking of angiotensin receptors has been shown to significantly reduce renal CaOx crystal deposition as well as the development of interstitial inflammation and the level of oxidative stress [80–82]. Finally, treatment with taurine, a naturally occurring sulfonic acid with renoprotective and anti-oxidant capacities, has been found to diminish crystal deposition in kidneys of the rat [83].

A *third* approach is to verify whether the crystal-binding molecules identified in vitro are also expressed in animal and human renal tissue. In this context, our laboratory (in collaboration with the laboratory of Experimental Urology of C. Verkoelen) investigated the tubular epithelial phenotypical changes during the development of intratubular nephrocalcinosis both in rats [42, 84] and in humans [26]. These studies not only demonstrated luminal expression of HA, OPN, ANX2 and NRP in kidneys with nephrocalcinosis, but also illustrated that alteration of the tubular epithelial phenotype can precede the onset of nephrocalcinosis. *First*, in EG-treated rats, CaOx crystals are readily formed and excreted within 24 h of EG-administration. Interestingly, both intratubular crystal retention and epithelial phenotypical changes were negligible at that time [42, 84], thereby corroborating the in vitro well-documented finding that a normal differentiated monolayer of renal epithelial cells does not bind crystals [43, 45]. *Second*, despite continuous crystal formation and excretion during a 4-day EG-administration period, crystal retention only gradually increased, in parallel with the extent of epithelial changes [84]. This suggests that crystal retention progresses at the rate at which the tubular epithelium is altered, rather than being solely dependent on the presence of crystals in the tubular fluid. *Third*, at 2 days after arrest of a 4-day EG-administration period, crystal retention markedly increased, while crystal excretion had completely stopped [84]. Although crystal formation in the tubular fluid appeared to have decreased at that time, the observation that the number of regenerating epithelial cells had clearly increased, suggested that, after withdrawal of the toxic pressure of EG (and its metabolites), the kidney fully unfolds its natural regenerating ability, leading to an increased presentation of dedifferentiated cells capturing nearly every crystal passing by [84]. *Finally*, in humans, by investigating the luminal expression of HA and OPN in renal tissue obtained from renal transplant patients and preterm infants at two consecutive time points, i.e. prior to and during intratubular nephrocalcinosis, it was found that these epithelial alterations precede the onset of intratubular nephrocalcinosis [26]. Interestingly, preterm infants, always born without nephrocalcinosis, may develop it after birth due to clinical treatment [29], suggesting that epithelial changes even precede the onset of crystal formation in the tubular fluid. This indicates that crystals are to be considered a

potential, but not a strictly necessary cause of the epithelial alterations known to be associated with intratubular nephrocalcinosis.

The tubular epithelium during the course of nephrocalcinosis

The effect of intratubular nephrocalcinosis on renal function, besides influencing tubular fluid flow, is further determined by the reaction of the tubular epithelium towards intraluminal immobilized crystals. Numerous in vitro studies show that exposure of the tubular epithelium to CaP and/or CaOx crystals induces the production of inflammatory mediators (MCP-1, PGE-2) and excess reactive oxygen species, and release of lactate dehydrogenase (LDH) [27, 67, 85–87]. These findings suggest that, once prolonged crystal–cell contact is established, crystal-induced tubulo-intersitial injury and inflammation may progress to permanent loss of tubular function. However, observations in several patient groups with nephrocalcinosis, i.e. preterm infants [88], children with hypophosphatasia and X-linked hypophosphatemic rickets [89], and patients with obesity-related jejunoileal bypass-induced enteric hyperoxaluria [90], show that, as soon as excessive delivery of crystal constituents like calcium, oxalate or phosphate to the tubular system is decreased or prevented, nephrocalcinosis (acute or chronic) tends to disappear from the kidney and renal function, if impaired, either improves or stabilizes. These intriguing observations suggest that the tubular epithelium, besides its putative undesirable role as crystal binder when dedifferentiated, may also present a crystal clearing role during the course of nephrocalcinosis.

Rat studies from our laboratory and De Bruijn et al. [91, 92] aimed at isolating this role by specifically following the morphological tubulo-interstitial changes during the recovery of EG-induced intratubular nephrocalcinosis, in the absence of continuous crystal deposition. It is well known that the tubular epithelium, in vitro, is capable of clearing crystals by endocytosis and subsequent lysosomal disintegration as long as crystal diameters are smaller than that of the epithelial host cells they contact [93–96] (Fig. 1). In our in vivo study, however, as intracellular crystals were not unequivocally observed, endocytosis by epithelial cells did not appear to be the main crystal clearing mechanism. Either dedifferentiated cells with crystal-binding properties are no longer capable to endocytose crystals or the size of the observed intratubular crystal deposits, which was generally larger than an individual cell, prevents this process to take place. Nonetheless, the presence of these larger deposits revealed an alternative and performant tubulo-interstitial crystal clearing mechanism, termed "exotubulosis" [91, 92] (Fig. 1).

The key aspect of "exotubulosis" involves epithelial coverage of the crystalline deposit by proliferation and migration of epithelial cells neighbouring the crystal attachment site (Fig. 1). Although CaOx crystals have been shown to induce cellular proliferation [68], it is remarkable that different crystal types, such as CaP, cystine [97], adenine [98] and melamine [99], can be overgrown as well [92]. This indicates that not the crystal type per se but, rather a conserved reaction of the tubular epithelium towards contact with a solid material drives this process. Most likely, as viable dedifferentiated epithelial cells, by default, strive to maximize cell–cell contact, overgrowth of crystals is intuitively the result of the natural ability of the tubular epithelium to restore epithelial integrity and functionality. After epithelial overgrowth, the basement membrane of the crystal deposition site degrades and crystals are directed to the interstitium (Fig. 1). At this time, the tubules are already resealed as the young epithelium, which covers the crystals, matures and produces a new basement membrane adjacent to the crystals. Once exposed to the interstitial environment, crystals are being degraded and dissolved. As crystal deposits actually are conglomerates of mineral material and an organic matrix, and in addition, can be dissolved in vitro in solutions with low pH, it is currently hypothesized that crystals are cleared by the combined and local action of proteases and proton secretion [92, 96]. Importantly, the identification of similar tubulo-interstitial structures in different human pathologies with nephrocalcinosis, i.e. acute phosphate nephropathy, transplant patients, preterm infants and patients with primary hyperoxaluria, provides strong evidence for the existence of an active renal post-adhesion crystal clearing mechanism, both in rat and in man [92].

As to whether and which cell types mediate these processes, monocytes, macrophages and multi-nucleated giant cells have been identified in the vicinity of interstitial crystals in EG-treated rats and glyoxylate-injected mice [100–102]. Although in our study only a moderate association with macrophages was observed, it is important to note that in general there was an increased association of leukocytes both with crystal containing tubules and interstitial crystals during the course of renal crystal clearance [92]. Whether, leukocyte infiltration is merely due to a local crystal-induced inflammation or whether they are actively recruited to participate in crystal clearance remains to be determined.

Conclusion

The role of the tubular epithelium in nephrocalcinosis appears to be dual. Whereas there is increasing evidence that its dedifferentiation is a prerequisite for retention of relatively small intratubular crystals, it is also capable of

translocating these crystals to the interstitium, where they are subsequently dissolved. In particular, the presence of an active renal crystal clearing mechanism puts our understanding of the course and progression of intratubular nephrocalcinosis into a new perspective. Whether a patient either or not develops renal insufficiency attributed to nephrocalcinosis may depend on the delicate balance between the extent, duration and rate of crystal deposition on the one hand and the efficiency of crystal clearance on the other hand.

References

1. Gwinner W, Suppa S, Mengel M, Hoy L, Kreipe HH, Haller H, Schwarz A (2005) Early calcification of renal allografts detected by protocol biopsies: causes and clinical implications. Am J Transplant 8:1934–1941
2. Katz ME, Karlowicz MG, Adelman RD, Werner AL, Solhaug MJ (1994) Nephrocalcinosis in very low birth weight neonates: sonographic patterns, histologic characteristics, and clinical risk factors. J Ultrasound Med 10:777–782
3. Mole DR, Tomson CR, Mortensen N, Winearls CG (2001) Renal complications of jejuno-ileal bypass for obesity. QJM 2:69–77
4. Evan AP, Lingeman JE, Coe FL, Shao Y, Parks JH, Bledsoe SB, Phillips CL, Bonsib S, Worcester EM, Sommer AJ, Kim SC, Tinmouth WW, Grynpas M (2005) Crystal-associated nephropathy in patients with brushite nephrolithiasis. Kidney Int 2:576–591
5. Evan AE, Lingeman JE, Coe FL, Miller NL, Bledsoe SB, Sommer AJ, Williams JC, Shao Y, Worcester EM (2008) Histopathology and surgical anatomy of patients with primary hyperparathyroidism and calcium phosphate stones. Kidney Int 2:223–229
6. Kok DJ (1996) Crystallization and stone formation inside the nephron. Scanning Microsc 2:471–484
7. Asplin JR, Parks JH, Coe FL (1997) Dependence of upper limit of metastability on supersaturation in nephrolithiasis. Kidney Int 6:1602–1608
8. Kok DJ (1997) Intratubular crystallization events. World J Urol 4:219–228
9. Tiselius HG (1997) Estimated levels of supersaturation with calcium phosphate and calcium oxalate in the distal tubule. Urol Res 2:153–159
10. Parks JH, Coe FL, Evan AP, Worcester EM (2009) Urine pH in renal calcium stone formers who do and do not increase stone phosphate content with time. Nephrol Dial Transplant 1:130–136
11. Evan AP (2009) Physiopathology and etiology of stone formation in the kidney and the urinary tract. Pediatr Nephrol
12. Tiselius HG, Hojgaard I (1999) Some aspects of the intratubular precipitation of calcium salts. J Am Soc Nephrol:S371–S375
13. Coe FL, Parks JH, Evan A, Worcester EM (2007) Pathogenesis and treatment of nephrolithiasis. In: Alpern RJ, Hebert SC (eds) Seldin and Giebisch's the kidney, 4th edn. Academic Press, Burlington, pp 197–1945
14. Renkema KY, Velic A, Dijkman HB, Verkaart S, van der Kemp AW, Nowik M, Timmermans K, Doucet A, Wagner CA, Bindels RJ, Hoenderop JG (2009) The calcium-sensing receptor promotes urinary acidification to prevent nephrolithiasis. J Am Soc Nephrol 8:1705–1713
15. Sayer JA, Carr G, Simmons NL (2004) Nephrocalcinosis: molecular insights into calcium precipitation within the kidney. Clin Sci (Lond) 6:549–561
16. Unuma K, Tojo A, Harada K, Saka K, Nakajima M, Ishii T, Fujita T, Yoshida K (2009) Autopsy report on pseudo-Bartter syndrome with renal calcification induced by diuretics and diet pills. BMJ Case Rep. doi:10.1136/bcr.12.2008.1380
17. Devuyst O, Pirson Y (2007) Genetics of hypercalciuric stone forming diseases. Kidney Int 9:1065–1072
18. Evan AP, Lingeman J, Coe F, Shao Y, Miller N, Matlaga B, Phillips C, Sommer A, Worcester E (2007) Renal histopathology of stone-forming patients with distal renal tubular acidosis. Kidney Int 8:795–801
19. Wagner CA, Geibel JP (2002) Acid–base transport in the collecting duct. J Nephrol:S112–S127
20. Lee CT, Chen HC, Lai LW, Yong KC, Lien YH (2007) Effects of furosemide on renal calcium handling. Am J Physiol Renal Physiol 4:F1231–F1237
21. Bobrowski AE, Langman CB (2008) The primary hyperoxalurias. Semin Nephrol 2:152–162
22. Robertson WG, Peacock M, Nordin BE (1969) Calcium crystalluria in recurrent renal-stone formers. Lancet 294(7610):21–24
23. Elliot JS, Rabinowitz IN (1980) Calcium oxalate crystalluria: crystal size in urine. J Urol 3:324–327
24. Finlayson B, Reid F (1978) The expectation of free and fixed particles in urinary stone disease. Invest Urol 6:442–448
25. Markowitz GS, Nasr SH, Klein P, Anderson H, Stack JI, Alterman L, Price B, Radhakrishnan J, D'Agati VD (2004) Renal failure due to acute nephrocalcinosis following oral sodium phosphate bowel cleansing. Hum Pathol 6:675–684
26. Verhulst A, Asselman M, De Naeyer S, Vervaet BA, Mengel M, Gwinner W, D'Haese PC, Verkoelen CF, De Broe ME (2005) Preconditioning of the distal tubular epithelium of the human kidney precedes nephrocalcinosis. Kidney Int 4:1643–1647
27. Khan SR, Shevock PN, Hackett RL (1992) Acute hyperoxaluria, renal injury and calcium oxalate urolithiasis. J Urol 1:226–230
28. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR (2003) The natural history of chronic allograft nephropathy. N Engl J Med 24:2326–2333
29. Schell-Feith EA, Kist-Van Holthe JE, Conneman N, van Zwieten PH, Holscher HC, Zonderland HM, Brand R, van der Heijden BJ (2000) Etiology of nephrocalcinosis in preterm neonates: association of nutritional intake and urinary parameters. Kidney Int 5:2102–2110
30. Schwarz A, Mengel M, Gwinner W, Radermacher J, Hiss M, Kreipe H, Haller H (2005) Risk factors for chronic allograft nephropathy after renal transplantation: a protocol biopsy study. Kidney Int 1:341–348
31. Kok DJ, Khan SR (1994) Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int 3:847–854
32. Robertson WG (2004) Kidney models of calcium oxalate stone formation. Nephron Physiol 2:21–30
33. Huggins CB (1933) Bone and calculi in the collecting tubules of the kidney. Arch Surg 1:203–215
34. De Albuquerque PF, De Paola D (1959) Experiments in urolithiasis: I. Histochemical studies. J Urol:345–350
35. Haggitt RC, Pitcock JA (1971) Renal medullary calcifications: a light and electron microscopic study. J Urol 3:342–347
36. King JS Jr (1967) Etiologic factors involved in urolithiasis: a review of recent research. J Urol 4:583–591
37. Oliver J, MacDowell M, Whang R, Welt LG (1966) The renal lesions of electrolyte imbalance. IV. The intranephronic calculosis of experimental magnesium depletion. J Exp Med 2:263–278
38. Morgenroth K Jr, Backmann R, Blaschke R (1968) On the forms of calcium oxalate deposits in the human kidney in oxalosis. Beitr Pathol Anat 4:454–463
39. Khan SR, Finlayson B, Hackett RL (1979) Histologic study of the early events in oxalate induced intranephronic calculosis. Invest Urol 3:199–202

40. Dykstra MJ, Hackett RL (1979) Ultrastructural events in early calcium oxalate crystal formation in rats. Kidney Int 6:640–650
41. Sarica K, Erbagci A, Yagci F, Bakir K, Erturhan S, Ucak R (2004) Limitation of apoptotic changes in renal tubular cell injury induced by hyperoxaluria. Urol Res 4:271–277
42. Asselman M, Verhulst A, De Broe ME, Verkoelen CF (2003) Calcium oxalate crystal adherence to hyaluronan-, osteopontin-, and CD44-expressing injured/regenerating tubular epithelial cells in rat kidneys. J Am Soc Nephrol 12:3155–3166
43. Bigelow MW, Wiessner JH, Kleinman JG, Mandel NS (1998) Calcium oxalate crystal attachment to cultured kidney epithelial cell lines. J Urol 4:1528–1532
44. Riese RJ, Mandel NS, Wiessner JH, Mandel GS, Becker CG, Kleinman JG (1992) Cell polarity and calcium oxalate crystal adherence to cultured collecting duct cells. Am J Physiol (2 Pt 2):F177–F184
45. Verkoelen CF, van der Boom BG, Houtsmuller AB, Schroder FH, Romijn JC (1998) Increased calcium oxalate monohydrate crystal binding to injured renal tubular epithelial cells in culture. Am J Physiol (5 Pt 2):F958–F965
46. Verhulst A, Asselman M, Persy VP, Schepers MS, Helbert MF, Verkoelen CF, De Broe ME (2003) Crystal retention capacity of cells in the human nephron: involvement of CD44 and its ligands hyaluronic acid and osteopontin in the transition of a crystal binding- into a nonadherent epithelium. J Am Soc Nephrol 1:107–115
47. Kleinman JG, Sorokina EA, Wesson JA (2010) Induction of apoptosis with cisplatin enhances calcium oxalate crystal adherence to inner medullary collecting duct cells. Urol Res
48. Verkoelen CF, van der Boom BG, Kok DJ, Houtsmuller AB, Visser P, Schroder FH, Romijn JC (1999) Cell type-specific acquired protection from crystal adherence by renal tubule cells in culture. Kidney Int 4:1426–1433
49. Verkoelen CF, van der Boom BG, Romijn JC (2000) Identification of hyaluronan as a crystal-binding molecule at the surface of migrating and proliferating MDCK cells. Kidney Int 3:1045–1054
50. Asselman M, Verhulst A, Van Ballegooijen ES, Bangma CH, Verkoelen CF, De Broe ME (2005) Hyaluronan is apically secreted and expressed by proliferating or regenerating renal tubular cells. Kidney Int 1:71–83
51. Sorokina EA, Wesson JA, Kleinman JG (2004) An acidic peptide sequence of nucleolin-related protein can mediate the attachment of calcium oxalate to renal tubule cells. J Am Soc Nephrol 8:2057–2065
52. Bigelow MW, Wiessner JH, Kleinman JG, Mandel NS (1997) Surface exposure of phosphatidylserine increases calcium oxalate crystal attachment to IMCD cells. Am J Physiol (1 Pt 2):F55–F62
53. Lieske JC, Leonard R, Swift H, Toback FG (1996) Adhesion of calcium oxalate monohydrate crystals to anionic sites on the surface of renal epithelial cells. Am J Physiol (1 Pt 2):F192–F199
54. Lieske JC, Norris R, Toback FG (1997) Adhesion of hydroxyapatite crystals to anionic sites on the surface of renal epithelial cells. Am J Physiol (2 Pt 2):F224–F233
55. Wiessner JH, Hasegawa AT, Hung LY, Mandel GS, Mandel NS (2001) Mechanisms of calcium oxalate crystal attachment to injured renal collecting duct cells. Kidney Int 2:637–644
56. Kohri K, Kodama M, Ishikawa Y, Katayama Y, Matsuda H, Imanishi M, Takada M, Katoh Y, Kataoka K, Akiyama T (1991) Immunofluorescent study on the interaction between collagen and calcium oxalate crystals in the renal tubules. Eur Urol 3:249–252
57. Yamate T, Kohri K, Umekawa T, Amasaki N, Amasaki N, Isikawa Y, Iguchi M, Kurita T (1996) The effect of osteopontin on the adhesion of calcium oxalate crystals to Madin–Darby canine kidney cells. Eur Urol 3:388–393
58. Yamate T, Kohri K, Umekawa T, Konya E, Ishikawa Y, Iguchi M, Kurita T (1999) Interaction between osteopontin on Madin-Darby canine kidney cell membrane and calcium oxalate crystal. Urol Int 2:81–86
59. Yamate T, Kohri K, Umekawa T, Iguchi M, Kurita T (1998) Osteopontin antisense oligonucleotide inhibits adhesion of calcium oxalate crystals in Madin-Darby canine kidney cell. J Urol 4:1506–1512
60. Kumar V, Farell G, Deganello S, Lieske JC (2003) Annexin II is present on renal epithelial cells and binds calcium oxalate monohydrate crystals. J Am Soc Nephrol 2:289–297
61. Carr G, Simmons NL, Sayer JA (2006) Disruption of clc-5 leads to a redistribution of annexin A2 and promotes calcium crystal agglomeration in collecting duct epithelial cells. Cell Mol Life Sci 3:367–377
62. Sorokina EA, Kleinman JG (1999) Cloning and preliminary characterization of a calcium-binding protein closely related to nucleolin on the apical surface of inner medullary collecting duct cells. J Biol Chem 39:27491–27496
63. Lieske JC, Leonard R, Toback FG (1995) Adhesion of calcium oxalate monohydrate crystals to renal epithelial cells is inhibited by specific anions. Am J Physiol (4 Pt 2):F604–F612
64. Aihara K, Byer KJ, Khan SR (2003) Calcium phosphate-induced renal epithelial injury and stone formation: involvement of reactive oxygen species. Kidney Int 4:1283–1291
65. Thamilselvan S, Khan SR, Menon M (2003) Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. Urol Res 1:3–9
66. Umekawa T, Chegini N, Khan SR (2002) Oxalate ions and calcium oxalate crystals stimulate MCP-1 expression by renal epithelial cells. Kidney Int 1:105–112
67. Umekawa T, Chegini N, Khan SR (2003) Increased expression of monocyte chemoattractant protein-1 (MCP-1) by renal epithelial cells in culture on exposure to calcium oxalate, phosphate and uric acid crystals. Nephrol Dial Transplant 4:664–669
68. Lieske JC, Walsh-Reitz MM, Toback FG (1992) Calcium oxalate monohydrate crystals are endocytosed by renal epithelial cells and induce proliferation. Am J Physiol (4 Pt 2):F622–F630
69. Kumar S, Sigmon D, Miller T, Carpenter B, Khan S, Malhotra R, Scheid C, Menon M (1991) A new model of nephrolithiasis involving tubular dysfunction/injury. J Urol 5:1384–1389
70. Xue YQ, He DL, Chen XF, Li X, Zeng J, Wang XY (2009) Shock wave induced kidney injury promotes calcium oxalate deposition. J Urol 2:762–765
71. Khan SR (2005) Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. Urol Res 5:349–357
72. Baud L, Ardaillou R (1986) Reactive oxygen species: production and role in the kidney. Am J Physiol (5 Pt 2):F765–F776
73. Baud L, Ardaillou R (1993) Involvement of reactive oxygen species in kidney damage. Br Med Bull 3:621–629
74. Andreoli SP (1991) Reactive oxygen molecules, oxidant injury and renal disease. Pediatr Nephrol 6:733–742
75. Abid MR, Razzaque MS, Taguchi T (2005) Oxidant stress in renal pathophysiology. Contrib Nephrol:135–153
76. Huang HS, Chen J, Chen CF, Ma MC (2006) Vitamin E attenuates crystal formation in rat kidneys: roles of renal tubular cell death and crystallization inhibitors. Kidney Int 4:699–710
77. Thamilselvan S, Menon M (2005) Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. BJU Int 1:117–126
78. Wassmann S, Laufs U, Muller K, Konkol C, Ahlbory K, Baumer AT, Linz W, Bohm M, Nickenig G (2002) Cellular antioxidant effects of atorvastatin in vitro and in vivo. Arterioscler Thromb Vasc Biol 2:300–305

79. Itoh Y, Yasui T, Okada A, Tozawa K, Hayashi Y, Kohri K (2005) Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. J Urol 1:271–275
80. Toblli JE, Ferder L, Stella I, De Cavanaugh EM, Angerosa M, Inserra F (2002) Effects of angiotensin II subtype 1 receptor blockade by losartan on tubulointerstitial lesions caused by hyperoxaluria. J Urol (4 Pt 1):1550–1555
81. Toblli JE, Ferder L, Stella I, Angerosa M, Inserra F (2001) Protective role of enalapril for chronic tubulointerstitial lesions of hyperoxaluria. J Urol 1:275–280
82. Antus B, Exton MS, Rosivall L (2001) Angiotensin II: a regulator of inflammation during renal disease? Int J Immunopathol Pharmacol 1:25–30
83. Li CY, Deng YL, Sun BH (2009) Taurine protected kidney from oxidative injury through mitochondrial-linked pathway in a rat model of nephrolithiasis. Urol Res 4:211–220
84. Vervaet BA, D'Haese PC, De Broe ME, Verhulst A (2009) Crystalluric and tubular epithelial parameters during the onset of intratubular nephrocalcinosis: illustration of the 'fixed particle' theory in vivo. Nephrol Dial Transplant 12:3659–3668
85. Schepers MS, Van Ballegooijen ES, Bangma CH, Verkoelen CF (2005) Crystals cause acute necrotic cell death in renal proximal tubule cells, but not in collecting tubule cells. Kidney Int 4:1543–1553
86. Escobar C, Byer KJ, Khaskheli H, Khan SR (2008) Apatite induced renal epithelial injury: insight into the pathogenesis of kidney stones. J Urol 1:379–387
87. Umekawa T, Iguchi M, Uemura H, Khan SR (2006) Oxalate ions and calcium oxalate crystal-induced up-regulation of osteopontin and monocyte chemoattractant protein-1 in renal fibroblasts. BJU Int 3:656–660
88. Saarela T, Vaarala A, Lanning P, Koivisto M (1999) Incidence, ultrasonic patterns and resolution of nephrocalcinosis in very low birthweight infants. Acta Paediatr 6:655–660
89. Auron A, Alon US (2005) Resolution of medullary nephrocalcinosis in children with metabolic bone disorders. Pediatr Nephrol 8:1143–1145
90. Dhar NB, Grundfest S, Jones JS, Streem SB (2005) Jejunoileal bypass reversal: effect on renal function, metabolic parameters and stone formation. J Urol 5:1844–1846
91. De Bruijn WC, Boeve ER, van Run PR, van Miert PP, de Water R, Romijn JC, Verkoelen CF, Cao LC, Schroder FH (1995) Etiology of calcium oxalate nephrolithiasis in rats. I. Can this be a model for human stone formation? Scanning Microsc 1:103–114
92. Vervaet BA, Verhulst A, Dauwe SE, De Broe ME, D'Haese PC (2009) An active renal crystal clearance mechanism in rat and man. Kidney Int 1:41–51
93. Lieske JC, Norris R, Swift H, Toback FG (1997) Adhesion, internalization and metabolism of calcium oxalate monohydrate crystals by renal epithelial cells. Kidney Int 5:1291–1301
94. Lieske JC, Swift H, Martin T, Patterson B, Toback FG (1994) Renal epithelial cells rapidly bind and internalize calcium oxalate monohydrate crystals. Proc Natl Acad Sci USA 15:6987–6991
95. Schepers MS, Duim RA, Asselman M, Romijn JC, Schroder FH, Verkoelen CF (2003) Internalization of calcium oxalate crystals by renal tubular cells: a nephron segment-specific process? Kidney Int 2:493–500
96. Grover PK, Thurgood LA, Fleming DE, van Bronswijk W, Wang T, Ryall RL (2008) Intracrystalline urinary proteins facilitate degradation and dissolution of calcium oxalate crystals in cultured renal cells. Am J Physiol Renal Physiol 2:F355–F361
97. Evan AP, Coe FL, Lingeman JE, Shao Y, Matlaga BR, Kim SC, Bledsoe SB, Sommer AJ, Grynpas M, Phillips CL, Worcester EM (2006) Renal crystal deposits and histopathology in patients with cystine stones. Kidney Int 12:2227–2235
98. Persy V, Postnov A, Neven E, Dams G, De Broe M, D'Haese P, De Clerck N (2006) High-resolution X-ray microtomography is a sensitive method to detect vascular calcification in living rats with chronic renal failure. Arterioscler Thromb Vasc Biol 9:2110–2116
99. Brown CA, Jeong KS, Poppenga RH, Puschner B, Miller DM, Ellis AE, Kang KI, Sum S, Cistola AM, Brown SA (2007) Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. J Vet Diagn Invest 5:525–531
100. de Water R, Noordermeer C, Houtsmuller AB, Nigg AL, Stijnen T, Schroder FH, Kok DJ (2000) Role of macrophages in nephrolithiasis in rats: an analysis of the renal interstitium. Am J Kidney Dis 3:615–625
101. de Water R, Noordermeer C, van der Kwast TH, Nizze H, Boeve ER, Kok DJ, Schroder FH (1999) Calcium oxalate nephrolithiasis: effect of renal crystal deposition on the cellular composition of the renal interstitium. Am J Kidney Dis 4:761–771
102. Okada A, Nomura S, Higashibata Y, Hirose M, Gao B, Yoshimura M, Itoh Y, Yasui T, Tozawa K, Kohri K (2007) Successful formation of calcium oxalate crystal deposition in mouse kidney by intraabdominal glyoxylate injection. Urol Res 2:89–99