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ARTICLE

Saeed R. Khan

Renal tubular damage/dysfunction: key to the formation of kidney stones

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Abstract Supersaturation is the driving force behind crystal formation in the kidneys. It can, however, result only in the formation of crystals which can be harmlessly expelled. For stone formation, crystals must form in the kidneys and be retained there, which is indeed a rare occurrence. Crystalluria is common while stone formation is not. Only pathological changes in the kidneys including renal injury and dysfunction can accomplish crystal retention. Lethal epithelial cellular injury promotes crystal nucleation, aggregation and retention. Sub-lethal injury or dysfunctional cells may produce ineffective crystallization modulators and localized areas of supersaturation in the interstitium. The former will affect crystallization in the urine while the latter may cause precipitation in the interstitium and development of Randall's plaques.

Keywords Randall's plaque · Nephrolithiasis · Calcium oxalate · Oxalate · Inflammation

Introduction

Urinary stones can form anywhere in the urinary tract, from kidneys to the bladder but in the industrialized and affluent countries, they are generally restricted to the kidneys. Calcium oxalate (CaOx) is the most common type of kidney stone. Kidney stones generally form attached to the renal papillary tips. Randall first emphasized the importance of renal papilla when he described minute tubular calculi and subepithelial calcium plaques within and on human renal papillae and suggested that these could serve as focal points for stone development [1]. He suggested that interstitial subepithelial deposits of calcium phosphate or calcium carbonate arising from pathological conditions of the renal papilla eroded through to the papillary surface forming a type I lesion. He further suggested that excessive urinary supersaturation in association with tubular cell death resulted in crystal deposition in the collecting ducts producing a type II lesion. Both types of lesions acted as foci for further stone growth in the pelvis or papillary ducts. Thus Randall proposed a theory in which both urinary supersaturation and renal tubular damage play a part in stone formation.

S. R. Khan
Department of Pathology and Center for the Study of Lithiasis, College of Medicine, University of Florida, Gainesville, FL, USA
E-mail: khan@pathology.ufl.edu

Supersaturation and crystallization of calcium oxalate

The formation of kidney stones or nephrolithiasis is a result of crystal formation in the kidneys. The driving force for crystallization is the development of supersaturation with respect to the precipitating salt [2]. Human urine is a complex solution containing not only calcium (Ca) and oxalate (Ox) but also other ions and macromolecules that can interact with Ca and/or Ox and modulate crystallization. Thus the urinary CaOx supersaturation depends not only on the concentration of Ca and Ox but also on the presence of ions such as citrate and magnesium. It also depends upon the presence of macromolecules such as many proteins and lipids [3], which can bind or form complexes with Ca and/or Ox. Any cellular dysfunction that can affect various urinary ions and other substances can also influence CaOx supersaturation and crystallization in the kidneys.

Crystal formation, particularly of calcium phosphate (CaP) and CaOx, within the urinary tract is widespread. Humans excrete millions of urinary crystals daily indicating at least transient development of supersaturation. However, few develop kidney stones, probably because either the crystals do not form in the kidneys or the crystals that form do not stay there. It has been suggested that with a transit time across the kidney of 5–10 min, residence time for the crystals to nucleate and grow large enough to be trapped [4] is not enough. The inner diameter of various segments of renal tubules

ranges from 15 to 60 μm [5]. The crystals of CaOx, growing at the rate of 1–2 μm/min, cannot grow bigger than a few microns and are therefore excreted with urine without causing any stone episode.

Even though the crystals cannot form without supersaturation, it is only one step in the process of stone formation. In order for stone to be formed, not only do the crystals need to be retained within the kidney but they should also be located at sites from where crystals can ulcerate to renal papillary surface to form a stone nidus. It is hypothesized that renal injury promotes crystal retention and the development of stone nidus on renal papillary surface [6]. In addition, renal epithelial injury supports crystal nucleation at lower supersaturation [7]. Persistent mild hyperoxaluria by itself or through crystallization of CaOx is injurious to the renal epithelium [8].

Crystal nucleation

Widespread CaP and CaOx crystalluria is an indication that human urine is sufficiently supersaturated with respect to these salts for their nucleation and adequate growth. However, in vitro and in vivo studies have shown that renal injury can promote crystallization of calcific crystals by providing substrates for their heterogeneous nucleation. Cell degradation following renal epithelial injury produces numerous membrane vesicles, which have been shown in vitro to be good nucleators of both CaP and CaOx. In vivo crystals seen in the renal tubules of hyperoxaluric rats are always associated with cellular degradation products [7, 9]. The stone matrix contains both membrane vesicles and lipids. Phospholipids of the cell membranes are proposed to help crystal nucleation [10]. Lipids isolated from the kidney stone matrix also promoted the nucleation of CaOx crystals. Interestingly, membranes of injured but intact cells also showed the capacity to nucleate CaOx crystals. Direct nucleation on cell surface can also promote crystal retention within the tubule [11].

Crystal retention

Crystals can reside in the kidneys by (1) formation in the renal interstitium, (2) attachment to the renal epithelial cells after their formation in the renal tubules, (3) not moving with the urinary flow and growing large enough to be trapped and (4) aggregating with other crystals and thus accreting mass.

Crystal formation in the interstitium

Based on the concentration profile of calcium and oxalate in the urine, tubular fluid and renal tissue, it was suggested that interstitium of the inner medulla had the highest Ox concentration and the best chance of being the primary nucleation site for CaOx [12]. In the absence of any convective flow in the interstitium, the crystals had unlimited time to grow and develop into a kidney stone. However, CaOx crystals have not been reported in the renal interstitium of idiopathic stone patients [1, 13]. Even when hyperoxaluria is experimentally induced in an animal, deposition of CaOx crystals begins in the renal tubular lumen [14]. CaOx crystals can, however, migrate from tubular location to the interstitium [6].

Calcium phosphate crystals are frequently seen in the renal interstitium [15]. They are most probably formed as a result of renal cellular dysfunction or pathology and when ulcerated to the papillary surface support stone formation.

Crystal attachment to renal epithelium

Animal model and tissue culture studies have provided the evidence for crystal retention within the kidneys by attachment to renal epithelial cells. Experimental induction of CaOx nephrolithiasis starts with hyperoxaluria followed by crystalluria and crystal deposition in the kidney [5, 6]. Hyperoxaluria alone triggers increased urinary excretion of enzymes such as *N*-acetyl-*β*-glucosaminidase, gamma-glutamyl transpeptidase and alkaline phosphatase, indicating epithelial injury [8]. Crystal deposition is associated with overt injury as indicated by cellular death and degradation. Morphological injury appears to be mostly confined to the epithelium of crystal containing renal tubules [14]. The degree of renal injury and the amount of crystal buildup are dependent upon the intensity and length of hyperoxaluria. Interestingly, human stone formers with mild hyperoxaluric display enzymuria of proximal tubular origin [16].

Further support for crystal adherence to injured epithelia comes from studies with epithelial lining of the rat bladder [17]. Exposure of the bladder epithelium, after removal of surface glycosaminoglycans by triton X100 or hydrochloric acid, to CaOx crystals promoted their adherence to the exposed surfaces. Since heparin treatment reduced crystal attachment to the injured epithelium it was concluded that sulfated moieties were involved in crystal adherence.

Tissue culture studies provided evidence not only for the crystal adherence to the renal epithelium but also the possible mechanisms involved. When primary cultures of inner medullary collecting duct cells were exposed to crystals of CaOx, uric acid or hydroxyapatite, crystals preferentially adhered to cells with impaired tight junctions [18]. It was concluded that crystals adhered to basolateral components, which moved to the cell surface after damage to the tight junctions. Recently, similar conclusions were made when MDCK-1 cell monolayers were first physically injured by removal of a strip of cells and then exposed to CaOx crystals. Crystals specifically adhered to residues on the growth substrate and surfaces

of injured and regenerating cells. It was concluded that both mature and immature cells surfaces express crystal-binding molecules but, while they are available on surfaces of immature cells, in mature cells these molecules become available only after injury [19]. These results strongly support the suggestions that epithelial damage promotes crystal adherence to the renal epithelium.

Molecules, which become available on cell surfaces on exposure to high Ox and CaOx crystals, include phosphatidylserine, CD44, osteopontin, hyaluronan [20–22]. All of them have been shown to promote crystal adherence to renal epithelial cell surfaces.

Crystals not traveling with the urinary flow

It is assumed that urinary flow through the renal tubules is laminar, in which case the flow velocity should be very small near the epithelium and may even be zero at the epithelium. Thus crystals near the epithelial surface would be traveling, if at all, at a much slower speed [23]. Crystal movement can also be influenced by their morphology because of the Stokes drag. Calcium oxalate crystallizes both as CaOx monohydrate and dihydrate, which have different morphologies and thus have different magnitudes of drag. Even the spherulitic form of calcium oxalate monohydrate is not a compact unit. Urine trapped between the crystallites would put a drag on crystal movement. Gravity will also have an effect upon particles traveling upward. There is also the possibility that urinary flow through the renal tubules is not laminar because urine in the papillary-collecting ducts is suggested to move as discreet boluses and is propelled by peristaltic waves that occur at regular time intervals.

In animal models of nephrolithiasis, crystals preferentially deposit at cortico-medullary junction where loops meet proximal tubules, in collecting ducts near renal fornices and at papillary tips, where there is a change in luminal diameter of the renal tubules [24]. In the cortico-medullary junction area, proximal tubules with wider luminal diameter meet slightly narrower thin loops of Henle. Distal tubules and collecting ducts have acute 70° angles and z-bends in renal fornices at the papillary base. Openings of the 60–100 μm diameter ducts of Bellini at the renal papillary tip are slit-like and only 7–23 μm wide. At these sites, changing luminal diameter would disturb the urinary flow and impede the movement of crystals. Perhaps these architectural characteristics of the mammalian kidneys play a role in the development of human stones on renal papillary tips and in the lower calices and fornices of the kidneys.

Thus it is theoretically possible for crystals to be retained by their size alone, but to date single crystals larger than 10–12 μm have not been reported from either kidneys or urine of humans or rats. Crystals of 5–10 μm have been seen attached to the renal epithelium of hyperoxaluric rats. It is possible that as a result of fluid or particle drag close to the tubule wall, crystal/cell contact is increased, facilitating both physiological and pathological responses of cells to the presence of crystals. This prolonged contact may actually promote crystal adherence to the renal epithelium.

Crystal aggregation

All models of CaOx nephrolithiasis concede that crystal aggregation is probably involved in crystal retention within the kidneys since aggregation of crystals can have a considerable effect on the particle size, and aggregated crystals are commonly found in urine and stones [5]. Although CaOx crystalluria is common in both stone formers and healthy people, stone formers excrete more crystal aggregates [25]. When precipitation is induced, urine from stone formers produces larger crystal aggregates. Stone formers' urine is less inhibitory of crystal aggregation, and reduction in aggregation inhibition is proportional to the severity of stone disease.

Animal model studies have shown that production of certain crystallization modulators such as osteopontin and bikunin is increased in kidneys with CaOx crystal deposits and become incorporated in the growing deposits. Ultrastructural examination of crystal aggregates in the kidneys, as well as urine, displays membranous cellular material closely associated with the crystals [10]. It is our understanding that cell debris, formed as a result of exposure to high concentration of Ox and CaOx crystals, collects with the crystals resulting in the formation of larger particles. Membrane lipids with properly aligned calcium-binding head groups bridge crystals together and promote crystal aggregation.

Cellular damage/dysfunction and production of crystallization modulators

When renal epithelial cells are exposed to oxalate ions and CaOx crystals, there is an increase in gene expression and production of several urinary macromolecules, which modulate the nucleation, growth, aggregation and retention of crystals in the kidneys [3, 26]. Some of them, such as OPN, have specific domains to interact with cell membranes, which may facilitate immobilization and promotion of crystal attachment. Almost all of the modulators are produced by the kidneys and excreted in the urine. Many signaling molecules such as protein kinase C (PKC), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) and transcription factors, such as NF-κB and activated protein-1 (AP-1), involved in the expression and production of macromolecules are activated by reactive oxygen species (ROS). ROS are produced when renal epithelial cells are exposed to Ox and/or CaOx crystals. Exposure to excessive Ox and CaOx crystals produces more ROS than can be dealt with by endogenous antioxidant defenses, thus inducing oxidative stress in the kidneys resulting in renal injury. Damage to crystallization modulator producing cells may cause a decrease in the

production of modulator or they may be defective. Development of oxidative stress in the kidneys as a decrease in the amount and activity of urinary crystallization modulators has been reported in human stone formers [27].

Formation of stone nidus and development of stone

Crystals can be retained at many sites in the kidneys through the size-enhancing process of aggregation and by attachment to the renal epithelium. How do the crystals present inside the kidneys evolve into stones attached to the renal papillary surfaces? Obviously, crystals deposited in the renal cortical tubules or inside tubules of the renal papilla cannot become a nidus for the stone formation. Studies of Randall's plaques in human kidneys have shown interstitial CaP deposits and intraluminal CaOx deposits in stone formers' kidneys. It has been suggested that in idiopathic stone formers, CaP deposits originate in the basement membrane of the Loops of Henle and from there continuously grow outward reaching the papillary surface [28]. The CaP deposits on papillary surface then become focal points for the development of CaOx kidney stones. Randall's own studies, as well as of others, described the involvement of renal pathology in the development of the plaque. Both human and animal model studies have shown renal inflammation in association with crystal deposits [29], which become surrounded by multinucleate giant cells and ED-1 positive monocytes and macrophages. Exposure of renal epithelial cells to CaOx as well as CaP crystals induces the production of monocyte chemoattractant protein-1 [30, 31].

The migration of macrophages to plaque site and development of inflammation are likely to play a significant role in the ulceration of subepithelial deposits to renal papillary surface leading to the formation of stone nidus. Cultured non-transformed macrophages release pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleuken-6 (IL-6) into the medium [32] in response to CaOx crystal binding and phagocytosis. TNF-α induces the transcription and expression of several matrix metalloproteinases (MMP). Activation of MMPs requires cleavage by proteases derived from inflammatory cells such as neutrophils and monocytes [33]. Thus macrophages play a significant role in MMP gene expression and their subsequent activation. MMPs are the main matrix degrading enzymes and considered to play significant role in the erosion of atherosclerotic

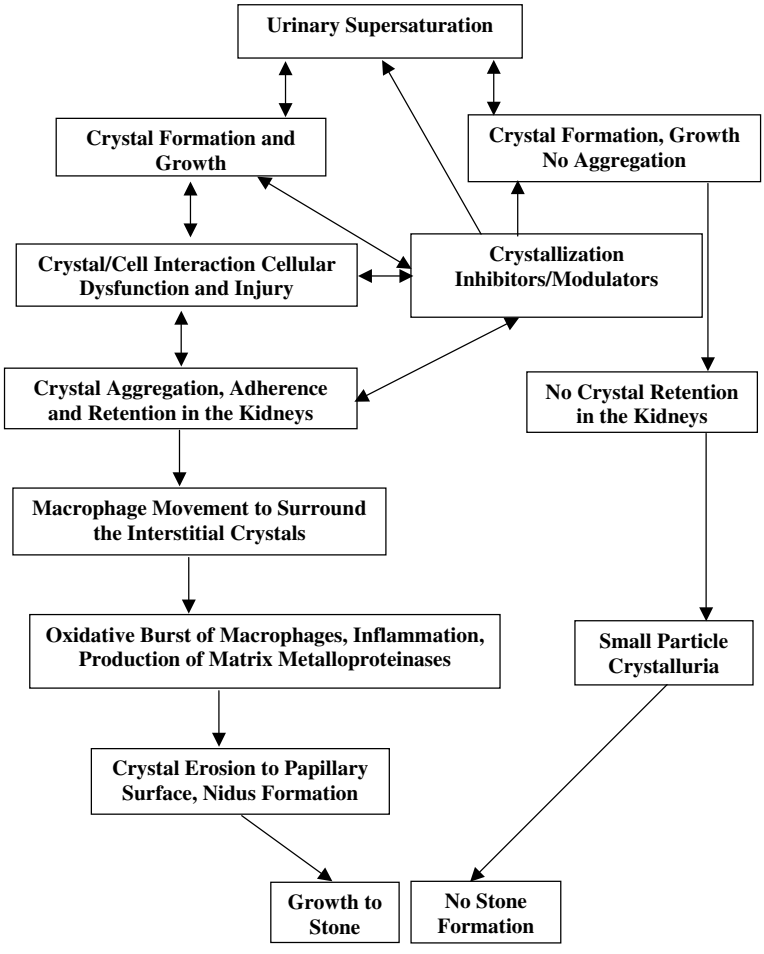

Fig. 1 Schematic presentation of relationships between various factors, which lead to the formation of idiopathic kidney stones. Urinary supersaturation controls crystallization but is itself controlled by the various consequences of crystal formation. Crystals that are not expelled with the urine induce production of crystallization modulators and may eventually lead to cellular dysfunction and degradation. Products of cell injury promote further crystallization, from heterogeneous nucleation to crystal aggregation and retention. Cell injury also promotes interstitial inflammation, which is likely involved in crystal erosion to papillary surface and the development of stone nidus

plaque [34] and may play similar role during stone formation in crystal erosion to renal papillary surface.

Concluding remarks

Figure 1 illustrates the relationships between various factors, which lead to the formation of kidney stones. The right side of the diagram illustrates that supersaturation by itself can only produce small particle crystalluria. The crystals do not aggregate, are not retained inside the kidneys and are expelled as crystalluria without causing the disease. It is only the pathological response of the kidney, as depicted in the left side of the diagram, which leads to stone formation. Double-headed arrows indicate the existence of interdependence. High supersaturation leads to crystal formation, which results in lowering of the supersaturation. Similarly crystal formation is necessary for crystal/cell interaction, cellular dysfunction and degradation while products of cell damage, either through membrane vesicles or surface exposure of specific molecules, promote crystallization. Renal cells respond according to the severity of the challenge. Response may be physiological, leading to the production of active crystallization inhibitors, or pathological, producing defective (?) inhibitors promoting crystal aggregation and adherence. In addition, damage to the cells may lead to both crystal nucleation and adherence. Crystallization modulators, both ionic and macromolecular, may affect supersaturation by binding calcium and/or oxalate (?). The most critical aspect of stone formation is migration of interstitial crystal deposits to the papillary surfaces, which is most likely directed by inflammatory cells and the production of metalloproteinases.

Supersaturation is considered necessary for the production of stones. Therefore reduction of supersaturation is the major goal of most therapies for stone formation. But supersaturation can only produce crystallization (Fig. 1). That is why in some cases highly supersaturated urine produces only crystalluria but no stones. One of the approaches to prevent stone formation would be to stop crystal retention. If crystals are not retained in the kidneys there will be no kidney stones. As discussed above, cellular damage promotes crystal retention through promotion of nucleation, aggregation and attachment of crystals to the renal epithelium. In addition, an inflammatory response to the crystals may be necessary for the development of the stone nidus.

Functional crystallization inhibitors are essential for the control of stone production. Damaged or dysfunctional state of the inhibitor producing cells may explain why stone formers produce insufficient and/or ineffective inhibitors. Since ROS appear responsible for cellular dysfunction/injury, control of renal oxidative stress may prove an effective therapy.

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References

1. Randall A (1940) The etiology of primary renal calculus. Int Abstr Surg 71:209
2. Kavanagh JP (1995) Calcium oxalate crystallization *in vitro*. In: Khan SR (ed) Calcium oxalate in biological systems. CRC, Boca Raton, p 1
3. Khan SR, Kok DJ (2004) Modulators of urinary stone formation. Front Biosci 9:1450
4. Finlayson B, Reid S (1978) The expectation of free or fixed particles in urinary stone disease. Invest Urol 15:442–448
5. Kok DJ, Khan SR (1994) Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int 46:847
6. Khan SR (1996) Calcium oxalate crystal interaction with renal epithelium, mechanism of crystal adhesion and its impact on stone development. Urol Res 23:71–79
7. Fasano JM, Khan SR (2001) Intratubular crystallization of calcium oxalate in the presence of membrane vesicles: an in vitro study. Kidney Int 59:169–178
8. Khan SR, Shevock PN, Hackett R (1989) Urinary enzymes and CaOx urolithiasis. J Urol 142:846–849
9. Khan SR, Shevock PN, Hackett RL (1990) Membrane-associated crystallization of calcium oxalate. Calcif Tissue Int 46:116
10. Khan SR, Glenton PA, Backov R, Talham DR (2002) Presence of lipids in urine, crystals and stones: implications for the formation of kidney stones. Kidney Int 62:2062
11. Lieske JC, Deganello S (1999) Nucleation, adhesion, and internalization of calcium-containing urinary crystals by renal cells. J Am Soc Nephrol 10:S422
12. Hautmann R, Osswald H (1983) Concentration profiles of calcium and oxalate in urine, tubular fluid and renal tissue—some theoretical considerations. J Urol 129:433
13. Khan SR, Finlayson B, Hackett RL (1984) Renal papillary changes in patient with calcium oxalate lithiasis. Urology 23:194
14. Khan SR, Hackett RL, Finlayson B (1981) Experimental calcium oxalate nephrolithiasis: role of renal papilla. Am J Pathol 10:59
15. Weller RO, Nester B, Cooke AAR (1971) Calcification in the human renal papilla: an electron microscopic study. J Pathol 107:211
16. Baggio B, Gambaro G, Ossi E, Favaro S, Boesatti A (1983) Increased urinary excretion of renal enzymes in idiopathic calcium oxalate nephrolithiasis. J Urol 129:1161
17. Khan SR, Cockrell CA, Finlayson B, Hackett RL (1984) Crystal retention by injured urothelium of the urinary bladder. J Urol 132:153
18. Riese RJ, Mandel NS, Wiessner JH, Mandel GS, Becker CJ, Kleinman JC (1992) Cell polarity and CaOx crystal adherence to cultured collecting duct cells. Am J Physiol 262:F177
19. Verkoelen CF, van der Broom BG, Houtsmuller AB, Schroeder FH, Romijn JC (1998) Increased CaOx monohydrate crystal binding to injured renal epithelial cells in culture. Am J Physiol 274:F958
20. Wiessner JH, Hasegawa AT, Hung LY, Mandel NS (1999) Oxalate-induced exposure of PS on surface of renal epithelial cells in culture. J Am Soc Nephrol 10:S441
21. Khan SR, Johnson JM, Peck AB, Cornelious JG, Glenton PA (2002) Expression of osteopontin in rat kidneys: induction during ethylene glycol induced calcium oxalate nephrolithiasis. J Urol 168:1173–1181
22. 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 CD 44 and its ligands hyaluronic acid and osteopontin in the transition from a crystal binding into a non adherent epithelium. J Am Soc Nephrol 13:107

23. Robertson WG (2004) Kidney models of calcium oxalate stone formation. Nephron Physiol 98:21
24. Khan SR (1996) Where do the renal stones form and how? Ital J Miner Electrolyte Metab 10:75
25. Robertson WG, Peacock M (1972) Calcium oxalate crystalluria and inhibitors of crystallization in recurrent renal stone-formers. Clin Sci 43:499
26. Khan SR (2004) Role of renal epithelial cells in the initiation of calcium oxalate stones. Nephron Exp Nephrol 98:e55
27. Huang H-S, Ma M-C, Chen C-F, Chen J (2003) Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. Urology 61:1123–1128
28. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, Shao Y, Sommer AJ, Paterson RF, Kuo RL, Grynpas M (2003) Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. J Clin Invest 111:607
29. Khan SR (2004) Crystal-induced inflammation of the kidneys, results of human, animal model and tissue culture studies. Clin Exp Nephrol 8:75–88
30. Umekawa T, Chegini N, Khan SR (2002) Oxalate ions and calcium oxalate crystals stimulate MCP-1 expression by renal epithelial cells. Kidney Int 61:105–112
31. 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 18:664–669
32. DeWater R, Leenen PJM, Noordermeer C, Nigg AL, Houtsmiller AB, Kok DJ, Schroeder FH (2001) Cytokine production induced by binding and processing of calcium oxalate crystals in cultured macrophages. Am J Kidney Dis 38:331
33. Sanders J-SF, van Goor H, Hanemaaijer R, Kallenberg CGM, Stegeman CA (2004) Renal expression of matrix metalloproteinases in human ANC-associated glomerulonephritis. Nephrol Dial Transplant 19:1412
34. van Osstrom O, Velema E, Schoneveld AH, de Vries JPPM, de Bruin P, Seldenrijk CA, de Kleijn DPV, Busser E, Moll FL, Verheijen JH, Virmani R, Pasterkamp G (2005) Age related changes in plaque composition, a study in patients suffering from carotid artery stenosis. Cardiovasc Pathol 14:126