Asian Journal of Urology 10 (2023) 246–257

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ajur

Review

Proposal for pathogenesis-based treatment options to reduce calcium oxalate stone recurrence

Saeed R. Khan [a,*], **Benjamin K. Canales** [b]

[a] *Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA*
[b] *Department of Urology, University of Florida, Gainesville, FL, USA*

Received 6 September 2022; received in revised form 2 December 2022; accepted 18 January 2023
Available online 13 April 2023

KEYWORDS
Kidney stones;
Calcium oxalate;
Inflammation;
Inflammasome;
Supersaturation;
Reactive oxygen species;
Oxidative stress

Abstract *Objective:* Prevalence of kidney stone disease continues to increase globally with recurrence rates between 30% and 50% despite technological and scientific advances. Reduction in recurrence would improve patient outcomes and reduce cost and stone morbidities. Our objective was to review results of experimental studies performed to determine the efficacy of readily available compounds that can be used to prevent recurrence.
Methods: All relevant literature up to October 2020, listed in PubMed is reviewed.
Results: Clinical guidelines endorse the use of evidence-based medications, such as alkaline agents and thiazides, to reduce urinary mineral supersaturation and recurrence. However, there may be additional steps during stone pathogenesis where medications could moderate stone risk. Idiopathic calcium oxalate stones grow attached to Randall's plaques or plugs. Results of clinical and experimental studies suggest involvement of reactive oxygen species and oxidative stress in the formation of both the plaques and plugs. The renin-angiotensin-aldosterone system (RAAS), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, mitochondria, and NOD-like receptor pyrin domain containing-3 (NLRP3) inflammasome have all been implicated at specific steps during stone pathogenesis in animal models.
Conclusion: In addition to supersaturation-reducing therapies, the use of anti-oxidants, free radical scavengers, and inhibitors of NADPH oxidase, NLRP3 inflammasome, and RAAS may prove beneficial for stone prevention. Compounds such as statins and angiotensin converting enzyme inhibitors are already in use as therapeutics for hypertension and cardio-vascular disease and have previously shown to reduce calcium oxalate nephrolithiasis in rats. Although clinical evidence for their use in stone prevention in humans is limited, experimental data support they be considered along with standard evidence-based medications and clinical expertise when patients are being counselled for stone prevention.

* Corresponding author.
E-mail address: khan@pathology.ufl.edu (S.R. Khan).
Peer review under responsibility of Tongji University.

https://doi.org/10.1016/j.ajur.2023.01.008

1. Introduction

Nephrolithiasis, formation of stones in the kidneys, is a common urological disorder. Prevalence of kidney stones appears to be increasing globally, and so is the cost associated with caring for patients with the disease [1,2]. Kidney stones recur at high rates [3], and are associated with maladies such as diabetes [4], hypertension [5], metabolic syndrome [6], and chronic kidney disease [7], suggesting that stones may even play a role in renal disease progression [7].

Kidney stones are aggregates of crystals mixed with a small amount of ubiquitous organic matrix. They are generally located in the renal calyces and/or pelvis and are classified by their most common crystalline constituent. Calcium oxalate (CaOx) mineral, often mixed with small amounts of calcium phosphate (CaP), constitutes up to 80% of all kidney stones worldwide [2]. Struvite, cystine, uric acid, and even some drugs or contaminants (such as melamine) are some of the other mineral stone types. Urinary mineral supersaturation leading to crystal formation and then retention/deposition within the kidneys are two major interrelated events of stone formation [8,9].

Great progress has been made in the surgical and medical management of nephrolithiasis [2]. Stones are surgically removed using percutaneous nephrolithotomy involving direct endoscopic access into the kidney through a small incision in the flank. Alternatively, retrograde intrarenal surgery is performed using a flexible fiberoptic ureteroscope to access the upper urinary tract. Larger stones are fragmented with a laser and removed with a basket, while fine portions of stone dust are allowed to pass naturally with the urine. Medical management includes therapies to facilitate stone expulsion and reduce stone recurrence, such as thiazide diuretics and alkalinizing medications. In addition, stone formers are advised to increase fluid intake (and thus urine output) as well as reduce dietary consumption of salt and animal protein [3]. Despite these advancements in treatment and prevention, stone recurrence continue to rise, with the most recent annual kidney stone incidence estimates at 2054 stones per 100 000 adults—or over 6 million kidney stones passed annually in the USA [3]. Why are rates so high? The therapies traditionally used to reduce stone formation (diet, medication, and fluid) focus on decreasing mineral supersaturation in order to be effective. Perhaps therapies that target the areas within the kidney where stones begin (plaques and plugs) are necessary to reduce stone recurrence and decrease stone expenditures. In this article, the current understanding of CaOx kidney stone pathogenesis based on clinical and experimental studies will be discussed as well as potential strategies to reduce recurrence through known stone pathogenesis pathways.

2. Clinical studies

2.1. Crystallization

More than 90% of a kidney stone is crystalline. As a result, major emphasis of clinical studies has been to determine the factors that control crystallization within the urine and the kidneys. Urinary supersaturation is driving force behind crystallization and is dependent upon a number of factors including urinary volume, calcium, oxalate, citrate, and urine pH. In addition, crystallization is influenced by a number of urinary macromolecules which can negatively or positively affect nucleation, growth, and aggregation of crystals as well as their retention within the kidneys [9,10]. Nucleation is the first stage in crystallization and, in the urine, most likely occurs heterogeneously [11], promoted by macromolecules, membrane fragments [10], or crystals of other mineral present in the urine, requiring lower level of supersaturation. Crystal growth is also dependent upon supersaturation and is likely influenced by modulators of crystallization present in the urine. *In vitro* studies have shown that magnesium, citrate, pyrophosphate, ADP, ATP, two phosphopeptides, various glycosaminoglycans, non-polymerized Tamm-Horsfall protein (THP) (also known as uromodulin), osteopontin (OPN), calgranulin, α1-microglobulin, β2-microglobulin, UPFT-1, and inter-α-inhibitor (bikunin, light chain), can all inhibit or retard crystal growth [10]. Conversely, other groups of modulators including matrix Substance A, various uncharacterized urinary proteins and glycoproteins, and the polymerized form of THP (uromucoid) have been shown to promote crystal growth [2].

Of many crystallization modulators shown experimentally to influence nucleation, growth, and aggregation, only citrate, magnesium, and pyrophosphate are considered to be clinically important in stone formation. Even though urinary supersaturation with respect to crystalline contents of stone is necessary for crystal formation and growth, it is unlikely to be solely responsible for stone formation, as this requires both crystallization and crystal retention within the kidneys. Analysis of supersaturation data from stone formers and non-stone formers also indicates that it alone cannot distinguish between urine samples of stone formers and non-stone formers [12,13].

2.2. Randall's plaques (RPs)

Idiopathic CaOx kidney stones are formed on renal papillae, in association with sub-epithelial plaques of CaP called RPs or on Randall's plugs (RPGs), which are crystalline deposits in the terminal collecting ducts [2,14,15]. Formers of RP associated stones, have abundance of plaque, fewer RPGs and produce more but smaller stones. Stone formers with

no apparent RP attachment show higher number of RPGs [15]. Plaques begin as interstitial deposits of CaP deep in the renal papillary tip and are proposed to start as concentrically laminated spherulitic CaP crystals in basement membrane of the loops of Henle. Mineralization proceeds from the basement membrane through the interstitium to the papillary urothelium. Collagen fibers, unidentified fibrillary materials, membrane-bound vesicles, and other cellular degradation products are mixed in with the CaP crystals [16]. OPN, heavy chain of inter-α-inhibitor [14,17], and zinc have also been identified in the plaque [18]. Ultrastructural examination of the renal papillae from primary hyperoxalurics [19] and idiopathic stone formers [16] show signs of injury and inflammation. One genome-wide gene expression study revealed considerable differences between papillary tissue of idiopathic CaOx stone formers and controls as well as differences between plaque and non-plaque papillary regions of stone formers. The expression of *LCN2*, *IL11*, *PTGS1*, *GPX3*, and *MMD* increased while that of *SLC12A1* and *NALCN* decreased in the renal papillary tissue associated with RP [20]. An increase in M1 macrophage related genes and a decrease in M2 macrophage related genes were seen in renal papillary tissue of stone patients [20,21]. Inflammatory cytokine, *CCL2*, *CCL5*, *CCL7*, CC-chemokine receptor 2 (*CCR2*), *CD40*, macrophage colony-stimulating factor 1 receptor (*CSF1*), CXC-chemokine ligand 9 (*CXCL9*), *CXCL10*, Fas ligand (*FASLG*), receptor-interacting serine/threonine kinase 2 (*RIPK2*), e-selectin (*SELE*), and Toll-like receptor 3 (*TLR3*), were also significantly increased [22].

2.3. RPGs

Plugs are formed when crystals are retained within the terminal collecting ducts and, unlike plaques, made of a variety of crystals. Plugs in primary hyperparathyroid stone patients are made of apatite. Apatite mixed with CaOx is seen in brushite stone formers, obesity bypass patients, patients with small bowel resection, and renal tubular acidosis. The plugs of ileostomy stone formers are made of apatite mixed with urate [23] and those of primary hyperoxaluria patients are made of CaOx [19,23]. Plugs, just like plaques, are also common in non-stone forming kidneys [24].

2.4. Organic matrix of idiopathic kidney stones

The stones contain a pervasive organic matrix containing carbohydrates, proteins, and lipids [25]. Crystals isolated from the human urine or produced *in vitro* in human urine also contain an organic matrix. Since Boyce first described Substance A in the stone matrix, investigators have been using ever more sophisticated techniques and number of unique proteins keeps increasing. One of the recent investigations reported 1059 unique proteins in stone matrix [26]. Some of the proteins are common to all stones while others are specific to stone types. In one study of 25 pure calcium stones, proteins involved in inflammatory processes were common in both CaOx and CaP stones [27], with roughly 1/3 proteins unique to CaOx, 1/3 unique to CaP, and the remainder were common in both. Calgranulin A and B, hemoglobin alpha- and beta-chains, fibrinogen, THP, alpha 1-antitrypsin, and vitronectin were found in over half the stones analyzed. Gene ontology analysis showed that CaOx monohydrate stone matrix contained more cell structure, cell membrane, intracellular transport proteins, and coagulative proteins while the matrix of apatite stones contained more immunity and defense proteins. Gene ontology analyses suggested that 61% had extracellular origin, 36% intracellular, and 3% intra- or extra-cellular. Ingenuity pathway analyses identified tumorigenesis, immunological disease, and inflammatory disease [28]. Matrix contained proteins of kidney, blood, and leukocyte origin [29]. Analysis of urine and stone matrix indicated that both contained proteins known to be involved in inflammation and fibrosis [30].

Microscopic investigations of intact and decalcified stones provided critical information about the location of matrix within the stones. Light and electron microscopic examination of decalcified CaOx stones showed the intimate relationship between the mineral and matrix [31]. Loss of crystals left only the matrix behind as crystal ghosts, which generally maintained the original shape of the crystals as well as stones. Tabular nature of the CaOx monohydrate, dipyramidal nature of CaOx di-hydrate, and spherical structure of the apatite was clearly visible. Stone interior showed concentric laminations and radial striations. Positive staining with Sudan Black B showed presence of lipids [32]. Ultrastructural immune-histochemistry showed the presence of OPN, THP, and albumin [33]. OPN was the most prominent constituent of stone matrix with intense labeling in the nucleus, concentric laminas, and radial striations of crystal ghost. Both THP and albumin were mainly present in the stone surface and not in direct contact with the CaOx crystals.

2.5. Epidemiology

Epidemiological data indicate that rates of both the incidence and prevalence of kidney stones are rising in nearly all countries across the globe [34]. In addition, gender, race, and geography also play significant role. Kidney stones used to be 2—3-times more common in men than women. However, recent data indicate that number of women suffering from kidney stones is increasing and male to female ratio is decreasing [35,36]. That race and ethnicity play a role in stone prevalence has also been recognized. For example, stone formation is more prevalent in non-Hispanic whites than Hispanic whites and African Americans [37]. Similarly South African whites have been shown to be more prone to stone disease than South African blacks [38]. However, critical analyses of the data indicate that race and ethnicity may not be as critical as perhaps diet and other factors [39]. Geographic location also plays a role in stone prevalence because it generally determines an individual's local environment. Hot and humid conditions lead to increased perspiration, insensate loss, decreased urinary volume, and thus, urine concentrated with calcium, oxalate, and other crystal promoting ions. Southeastern United States is called "stone belt" where hot and humid climate favors stone formation [40].

Kidney stones have also been associated with systemic diseases such as hypertension [5], diabetes [4], and metabolic syndrome [6]. Patients suffering with these diseases are at risk of stone formation, and stone formers are at risk of developing hypertension [7,41], chronic kidney disease, and even kidney failure. Epidemiological studies have also suggested an association between risk of cardiovascular diseases and history of kidney stones [5,42]. Kidney stone formation is associated with arterial calcification [43], and an increased risk of coronary heart disease [44]. There are, however, gender-based differences in the risk. Women with history of stones showed a modest increased risk of cardiovascular diseases [45].

2.6. Nutrition

Diet plays a crucial role in modifying various risk factors for stone formation [46,47]. Water intake directly affects urinary volume and thus concentration of both the promoting as well as inhibiting factors. Dietary intake of oxalate and its precursors such as ascorbic acid (vitamin C) influences urinary excretion of oxalate and thus crystallization in the urine [48]. Excretion of oxalate is also regulated by gut microbiota [49]. *Oxalobacter formigenes* (*O. formigenes*) was the first oxalate degrading bacterium isolated from the rumen of mammals as well as intestine of humans [50]. Correlation between the absence of *O. formigenes* and CaOx kidney stones has been reported [51,52]. Recent studies have shown that *O. formigenes* is part of a network of bacteria, which are necessary for healthy oxalate homeostasis [49,53]. A number of human trials have been performed to investigate the efficacy of treatment with oxalate degrading bacteria and oxalate degrading enzymes [54]. Trials of many promising therapies are also underway. In addition, to lower oxalate degrading capacity, patients with recurrent kidney stones have a dysbiotic gut microbial community and reduced butyrate-producing species, which are responsible for intestinal epithelial integrity and thus oxalate absorption. The alteration of gut microbiome, perhaps through dietary modifications, may be helpful in reducing stone recurrence [55].

High protein diet provides an acid load causing an increase in urinary calcium and uric acid and decrease in urinary pH and citrate, promoting crystallization [56,57]. Moderate dietary restriction of protein in patients with idiopathic hypercalciuria and calcium nephrolithiasis has beneficial outcomes [58]. High sodium intake also increases urinary excretion of calcium and low salt diet is effective in reducing urinary calcium [59]. A normal dietary calcium intake is necessary in stone formers because the calcium is thought to bind dietary gut oxalate, reducing its absorption and subsequent excretion in the urine [60].

Diet also affects production of reactive oxygen species (ROS) and inflammation, common features of stone formation, and various co-morbidities discussed earlier. One epidemiologic study showed that a diet (the Dietary Approaches to Stop Hypertension or DASH diet), reduces oxidative stress, improves vascular function, and lowers blood pressure [61], reducing not only stroke and cardiovascular disease risk but also the risk for stone formation by up to 45% [62]. Lower serum levels of alpha- and beta-carotene have been found associated with history of kidney stones [63]. Statins prescribed for lowering cholesterol, and inhibiting the production of ROS, not only reduces cardiovascular episodes [64,65], but also lowers the risk of stone formation [66]. A retrospective study that found statin use was protective against urolithiasis also reported the concomitant use of thiazides in their cohort, which may confound the statin finding [67]. Despite the uncertainty of statins in kidney stone prevention, there is evidence that statin use may contribute to reduced stone risk in certain patient populations.

2.7. Androgen receptor (AR)

An association between idiopathic kidney stones and testosterone has long been suspected. Both male and female CaOx stone formers have high serum testosterone compared to healthy volunteers [68—70]. The AR has been shown to be upregulated in male CaOx kidney stone formers [71]. CAG repeats and intron 4 C/G polymorphism is shown to be associated with kidney stones [72].

2.8. Urinary microbiome

Results of a number of recent clinical studies described significant differences in urinary microbiome between stone formers and non-stone formers [55,73]. There was lower diversity of urinary microbiota and differential representation of inflammation associated bacteria in male CaOx stone formers compared to normal controls [74]. Comparative functional analysis of urinary tract microbiome revealed reduced levels of genes associated with oxalate metabolism, transmembrane transport, proteolysis, and oxidation—reduction processes in CaOx stone formers [75]. Microbiome and metabolomic profiles showed significant differences between stone formers and non-stone formers [76]. Microbial composition of male and female stone formers urine was also significantly different. Thirty-nine taxa were differentially expressed between control and stone patients. One hundred and twelve metabolites were also differentially expressed. Future studies are needed to determine the possibility of altering urobiome.

3. Experimental studies

Animal models and tissue culture studies have been performed to understand crystallization in the kidneys and how it affects the normal structure and function of the kidneys.

3.1. Animal models

A variety of animal models utilizing mice, rabbits, rats, pigs, or drosophila have been developed to study *in vivo* the pathogenesis of CaOx kidney stones. Rats and mice (murine models) are the two most commonly used experimental animals for studying stone disease [77]. Despite their popularity, rodent kidneys are small, uni-papillate, and have fewer urinary tubules than the larger, multi-papillate kidneys of humans. Administration of oxalate as sodium or

ammonium oxalate or oxalate precursors [78] such as ethylene glycol, hydroxy-L-proline, glyoxylate or glycolate have generally been utilized to induce hyperoxaluria, CaOx crystalluria, and nephrolithiasis. Degree of hyperoxaluria produced is much higher than seen in idiopathic stone formers. It can either be induced alone or in combination with hypercalciuria. Hypercalciuria and CaP crystal deposition can be induced through dietary or genetic manipulations. Crystals, CaOx as well as CaP, are generally luminal and are see in both the proximal and distal tubular locations.

The hyperoxaluria and CaOx crystal deposition lead to many structural and pathophysiological changes in the kidneys altering urinary composition [79,80]. Kidney is under oxidative stress. Epithelial cells are damaged and are sloughed off into the urine. Monocytes and macrophages migrate around tubules with crystal deposits. These renal responses are facilitated by ROS. Lipid peroxides increased in both the renal tissue and urine in rats with hyperoxaluria and CaOx nephrolithiasis. Treatment with vitamin E improved tissue levels of antioxidant enzymes, reduced peroxidative tissue injury, and totally eliminated CaOx crystal deposition in the kidneys [81]. Total renal cellular glutathione is decreased and lipid peroxides are increased. The angiotensin II receptor antagonist losartan, known to reduce oxidative stress, produced significant increase in glutathione concentration and decrease in thiobarbituric acid reactive substances in the kidneys. Activities of catalase and superoxide dismutase increased in kidneys while alpha- and mu-glutathione-S-transferase levels increased in the urine of hyperoxaluric rats [82]. Production of ROS is in part mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and rennin angiotensin system. Reduction of angiotensin production or blocking the angiotensin receptor, reduces CaOx crystal deposition associated inflammatory responses [83]. Inhibition of NADPH oxidase by apocynin, an organic compound in the vanillin family that prevents production of superoxide in neutrophils in humans, reduced ROS production and CaOx crystal deposition in a rodent model of hyperoxaluria [80]. Atorvastatin, a competitive inhibitor of HMG-CoA reductase which reduces serum levels of triglycerides and high-density lipoprotein cholesterol, reduced the expression of Nox-1 and p22phox subunits of NADPH oxidase as well as inhibited crystal deposition in animal models [84].

ROS also activate inflammasomes involved in the proteolytic activation of proinflammatory cytokines interleukin-1 beta and interleukin-18 [85,86]. The nucleotide binding oligomerization domain-like receptor family, NOD-like receptor pyrin domain containing-3 (NLRP3) inflammasome mediates interleukin-1 beta secretory pathway and crystal induced inflammation in the kidneys [87,88]. CaOx crystals activate NLRP3 inflammasome via ROS induced thioredoxin-interacting protein (TXNIP) pathway [89]. This pathway is stopped by apocynin, an inhibitor of NADPH oxidase. Atorvastatin reduces oxidative stress and CaOx crystal deposition in rat kidneys by inhibiting NLRP3 inflammasome [90].

Since males are more prone to stone formation, a number of studies have investigated the involvement of AR in the process. Induction of hyperoxaluria in mice that have selective knock out of AR showed that hepatic AR deficient mice produce less oxalate than controls. Mice that lack renal tubular AR have marked reductions in CaOx crystal deposition within the kidney. Results showed direct involvement of AR in upregulation of hepatic glycolate oxidase and renal epithelial p22-PHOX unit of NADPH oxidase at the transcription level. AR degradation with ASC-J9® resulted in reduced CaOx crystallization within the kidneys [91]. Mice with renal tubular specific AR knockout have less CaOx crystals and more M2 macrophages compared to the wild type mice. AR expression in renal tubular epithelial cells in a rat model of hydroxy proline induced hyperoxaluria with small molecule ASC-J9® led to a decrease in CaOx crystal deposition and increased recruitment of macrophages or M2 polarization [92].

Deficiency of NLRP3 or ASC causes significantly less CaOx crystal deposition, tubular injury, and kidney injury molecule-1 in mice fed with high oxalate diet compared to wild-type mice. Similar results were obtained by inhibiting NLRP3 inflammasome. In addition, inflammasome inhibition produced infiltrates with anti-inflammatory M2c macrophages while controls showed proinflammatory M1 and profibrotic M2a-like macrophages. Results indicate the inflammasome involvement in regulating macrophage infiltration [93].

Low pyrophosphate levels caused by mutation in *ABCC6* gene lead to progressive soft tissue calcification. Abcc6$^{-/-}$ mice spontaneously develop interstitial apatite deposition, similar to the original apatite deposition during the formation of RPs [94].

Renal expression of many known crystallization modulators including OPN [83], inter-alpha-inhibitor, alpha 1-microglobulin [95], calgranulin, heparin sulfate, and matrix Gla protein (MGP) [96], is increased in hyperoxaluric rats. Expression of NFκB, kidney injury molecule, proliferating cell nuclear antigen, CD44, and E-cadherin is increased in renal tubules, particularly in association with crystal deposits [80]. Urinary excretion of many of these molecules is also increased [97]. CaOx crystal deposition is also associated with upregulation of the genes encoding for some crystallization modulators including alpha 1-microglobulin/bikunin precursor (*Ambp*), fibronectin-1 (*Fn-1*), fetuin (*Fetub*), hyaluronan synthase (*Has1*), osteopontin (*Opn*), matrix Gla protein (*Mgp*), and CD44 (*CD44*), whereas some encoding genes are down-regulated, such as prothrombin/coagulation factor 2 (*F2*), Tamm-Horsfall protein/uromodulin (*Umod*), calgranulin-N (*S100a9*), and inter-α-trypsin inhibitor heavy chains-1,3,4 (*Itih1, Itih3*, and *Itih4*), as well as heparanase (*Hpse*) [98]. Apocynin, the inhibitor of NADPH oxidase and an antioxidant reverses the effect of hyperoxaluria [99]. CaOx crystal deposition is reduced in OPN knockout mice with experimentally induced hyperoxaluria. Similarly, inactivation of THP gene in mice significantly increases severity of CaOx crystal deposition [100]. A percentage of OPN and THP knockout mice develop interstitial CaP deposits in their kidneys [100,101].

Hyperoxaluria in rats also leads to osteogenic changes in the kidneys. Genome wide analyses of differentially expressed genes consistent with dedifferentiation of epithelial cells into osteogenic phenotype showed that experimentally induced hyperoxaluria in male rats leads osteogenic changes in the kidneys [99]. Expression of genes encoding for runt related transcription factors (RUNX1 and

RUNX2), zinc finger protein Osterix, bone morphogenetic proteins (BMP2 and BMP7), bone morphogenetic protein receptor (BMPR2), collagen, osteocalcin, osteonectin, OPN, MGP, osteoprotegerin, cadherins, fibronectin, and vimentin is increased. Expression of genes encoding for alkaline phosphatase and cytokeratins 10 and 18 is decreased. Interestingly osteogenic changes are not associated with the deposition of CaP mineral, perhaps because of the downregulation of alkaline phosphatase and upregulation of OPN and MGP.

3.2. Tissue culture

Crystallization within the kidneys brings crystals in contact with the renal cells, most probably tubular epithelial cells. Thus, *in vitro* studies are performed to understand the consequences of these interactions. Most studies involved exposure of renal epithelial cells to CaOx or CaP crystals in culture. Interaction leads to increased expression of genes that encode for transcriptional activators, regulators of the mineralization modulators, growth factors, and production of molecules such as OPN, monocyte chemoattractant-1 (MCP-1), prostaglandin E2, components of inter-α-inhibitor including bikunin, alpha 1-microglobulin, CD44, calgranulin, heparin sulfate, osteonectin, fibronectin, and MGP [83,97,102]. Many of these molecules have roles in both mineralization and inflammation and fibrosis [10].

Studies show that cells exposed to CaOx or CaP crystals are injured and produce ROS [103] with the involvement of both mitochondria [104,105] and NADPH oxidase [97,102,106]. Production of ROS and cell injury can be stopped by antioxidants, free radical scavengers, and inhibitors of NADPH oxidase [102,104,107]. Brushite induced lactate dehydrogenase release and production of hydrogen peroxide and gamma inducible protein by LLC-PK1 and MDCK cells was reduced by catalase. Exposure of the HK-2 cells to oxalate or CaOx crystals increases the MCP-1 mRNA as well as protein which is significantly reduced by pre-treatment with catalase and superoxide dismutase [108].

One of the major sources of ROS production in the kidneys is NADPH oxidase, which shows increased activity when HK-2 cells are exposed to oxalate and CaOx crystals. There is an increase in the expression of various sub-units of NADPH oxidase, with consistent increases in the expression of membrane bound $p22^{phox}$ and cytosolic $p47^{phox}$. The production of superoxide and 8-isoprostane and lactate dehydrogenase release are increased [106]. Cellular response to oxalate is mediated by Rac-1 and PKC-alpha- and -delta-dependent activation of NADPH-oxidase [109]. Expression of OPN and MCP-1 induced by exposure to CaOx, brushite, or uric acid was reduced by treatment with diphenyleneiodium (DPI), a NADPH oxidase inhibitor [102].

Mitochondria are significant sites of CaOx, crystal-induced superoxide production, and glutathione depletion in renal epithelial cells in culture. Isolated mitochondria reacted to oxalate exposure by accumulating ROS, lipid peroxides, and oxidized thiol proteins [110]. The addition of C-phycocyanin provided protection against oxalate induced injury of the MDCK cells resulting in increased cell viability and reductions in production of ROA and lipid peroxidation. C-phycocyanin also repaired and restored mitochondrial trans-membrane potential [111]. Similarly, pretreatment of NRK-52E cells with MitoSOX Red, an antioxidant targeting mitochondria, restored mitochondrial membrane potential and increased cell viability [112]. High oxalate and CaOx crystals also induced mitochondrial dysfunction in human monocyte derived cell line disturbing redox homeostasis [65], which may interfere with crystal clearance by the monocytes. CaOx and other crystal deposits surrounded by macrophages are commonly encountered in human kidneys as well as kidneys of hyperoxaluric rats [33]. Clearance of crystals is likely caused by monocyte differentiating into M2 macrophages [113].

Sirtuin 3 (SIRT3) is a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase acting in the mitochondria to decrease ROS production and improve respiration. Kidneys from human CaOx stone formers showed enhanced apoptosis and downregulated SIRT3 expression. Compared to controls, hyperoxaluric mice that over-expressed SIRT3 had less epithelial injury, fewer CaOx crystal deposits, and decreased inflammatory macrophage infiltrates within renal parenchyma. [114,115]. This protective role of SIRT3 is suggested to be through promotion of macrophages towards M2 phenotype.

It has been suggested that CaOx crystals cause mitochondrial dysfunction via opening of mitochondrial permeability transition pores. The mitochondrial permeability transition pore opening depends upon the crystal induced activation of cyclophilin D of mitochondrial by NADPH oxidase produced ROS. Cyclosporine A, a calcineurin inhibitor and immunosuppressant medication, has been shown to inhibit this process [66].

4. Pathogenesis and therapy

Kidney stones are an end product of a series of events involving a variety of factors, some of which are specific to a stone type while others are universal for all kinds of stones. Since crystallization is a common factor for all stones, reducing supersaturation with respect to the stone crystals and thus increasing urinary volume is central to all therapies. Increasing urinary output is also necessary to reduce urinary oxalate and calcium and formation of CaOx crystals. In addition, reduction of dietary oxalate intake and cessation of supplements like vitamin C reduce urinary oxalate excretion. Modifications are needed for the reduction in oxalate excretion requiring low oxalate diet and stopping certain supplements such as vitamin C (ascorbic acid). Although hypercalciuria is a common feature of kidney stone formers, dietary reduction of calcium is not advised, as a diet low calcium can allow for increased gut absorption of oxalate leading to its increased urinary excretion. However, sodium consumption is to be reduced because it has an effect on urinary excretion of calcium. Hypocitraturia, a risk factor for stone recurrence, can be ameliorated by increased citrate intake by pill form (potassium citrate and sodium bicarbonate) and/or increased ingestion of citrus fruits or juices.

Crystallization in the kidneys is only one aspect of stone disease. Crystals can form in the renal tubules and urine, and be excreted with it. For stone formation, crystals have to be retained in the kidney. As presented earlier, there are

two ways for crystal retention. The first pathway is through RP formation, which originates in the interstitium and grows outward by addition of more CaP crystals. Eventually, these crystals reach the papillary surface and become exposed to pelvic urine, acting as a platform for nucleation/deposition of CaOx/CaP crystals. The second pathway is through crystal aggregation until the mass is large enough to block terminal collecting ducts. These "Randall's plugs" then act as nidus for further addition of crystals to grow into stones. How should these stone recurrence that developed on plaques or plugs be prevented? At present, when and where a stone will develop on these plaques or plugs is not known. Autopsy studies show that non-stone formers with normal kidneys have interstitial crystal deposits and plaque without stones [2]. Endoscopic papillary studies of stone formers show that most plaques (>90%) do not have attached stones. What causes stones to develop on one plaque or plug while avoiding others? And what causes stones attached to plaque to break off while others stay attached? These are clinical questions that remain unanswered.

Regardless of how stones overgrow and form on renal deposits, the basic science knowledge of stone pathogenesis should be used to develop strategies to lower stone recurrence. It is well established now that high oxalate and CaOx crystals are injurious to the exposed renal epithelial cells—an injury that can result in cell death. Urine is normally metastable with respect to CaOx, but the addition of these dead cells, along with other cellular degradation products in the urine, can promote heterogenous nucleation of crystals. Injury also provides sites for crystal adherence. Plug formation most probably begins with the formation of crystals within the tubules, perhaps as early as the proximal tubules, as has been suggested. Moreover, cells of the proximal tubular epithelium are more susceptible to oxalate and crystals. These crystals are small and easily move down with the urine. There are 1—1.5 million renal tubules in human kidney, and their contents eventually empty into a very small number of terminal collecting ducts. Crystals which have thus far been easily moving down the nephrons are suddenly massed together, aggregating and coming in direct contact with the epithelium. Aggregation and injury to the epithelium can promote crystal retention and plugging of the ductal openings. Obviously, the best strategy to prevent stone formation on plugs is to reduce supersaturation (as discussed above). In addition, renal injury associated with hyperoxaluria should be avoided through dietary modifications and/or pharmaceuticals. Production of ROS is a major source of renal injury in CaOx nephrolithiasis as well as co-morbidities such as obesity, chronic kidney disease, hypertension, metabolic syndrome, and diabetes [116]. Thus, medication which can control or even lower the development of oxidative stress in the kidneys would be expected to improve stone outcomes in this area [104].

Pathogenesis of stones developed over a plaque is more complicated. First there is the formation of plaque, which is followed by its growth and eventual exposure to the pelvic urine. Once plaque is exposed to urine, then supersaturation becomes important and current therapies which are based upon reducing excretion of calcium and oxalate and increasing that of citrate along with proper changes in the pH should be followed. However, supersaturation-based therapies are not fully able to stop recurrences with roughly 50%—70% of patients continuing to form stones. Perhaps a strategy that reduces plaque formation, its growth and exposure to the pelvic urine, and/or even elimination of the plaques would be beneficial. Results of recent studies, both clinical as well as experimental, reviewed here and elsewhere, suggest immunological response by the kidneys to various stone risk factors, particularly high oxalate and CaOx crystals. There is development of oxidative stress, injury, and inflammation. Activation of renin-angiotensin-aldosterone system and NADPH oxidase, causes an increase in the production of ROS. The ROS regulate the production of molecules playing roles in both crystallization as well as inflammation. The activation of NLRP3 inflammasome causes the production of caspases and inflammatory cytokines that promote macrophage infiltration. Normally, the presence of crystallization inhibitors keeps crystal number and crystal sizes in check, and these are easily passed during urination. Larger crystals are either taken in by the epithelial cells, destroyed by the phagocytic mechanism, or extruded into the interstitium where infiltrating macrophages eliminate them. This elimination process by macrophages is dependent upon macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 type. M1 macrophages are also implicated in vascular plaque calcification while M2 with plaque regression [64,117]. In addition to macrophage infiltration, inflammation also includes deposition of collagen. Collagen mineralization helps RP grow outward. Disruption of the tight junctions of papillary surface urothelium by metalloproteases (MMPs) may be responsible for uncovering of the plaques and their exposure to the pelvic urine, similar to what happens in plaque rupture during myocardial infarction [118]. Most significant aspect of plaque pathogenesis is, that a number of significant events are regulated by the production of ROS and as discussed above, have experimentally been shown to be reduced by specific anti-oxidants or free radical scavengers. MMPs are also activated by ROS [119]. Specific inhibitors of MMPs [120], NADPH oxidase [121], and inflammasome [122], are also currently available. Small molecule ASC-J9®, that degrades AR leading to reduction in CaOx crystallization and increase in M2 polarization is another possible drug.

5. Future perspectives

Hypercalciuria, hyperoxaluria, hypocitraturia, and low urine volume are the primary factors responsible for increasing mineral supersaturation and CaOx stone formation. As a result, calcium, citrate, and oxalate are the main targets of therapeutic modifications, by reducing dietary oxalate, salt, and protein and increasing dietary citrate. Therapies using oxalate degrading bacteria and oxalate degrading enzymes isolated from certain bacteria or fungi are presently being tested to treat primary hyperoxaluria. Infusing RNA interference to shifting enzymatic liver pathways has recently been shown to improve outcomes in patients with genetic disorders of primary hyperoxaluria [123].

Oxalate not only plays a major role in the development of mineral supersaturation and crystallization but, by itself, can

be injurious to the renal epithelium. Hyperoxaluria and its associated crystal deposition in the kidney are associated with the production of ROS most probably with the involvement of renin-angiotensin-aldosterone system (RAAS), mitochondria, and NADPH oxidase. These ROS then activate NLRP3 inflammasomes leading to inflammation and injury. Thus, reducing ROS production or their scavenging could be one therapeutic approach to decrease stone risk. The antioxidants, free radical scavengers, and inhibitors of active participants may be helpful and should be investigated for their ameliorating potential. Some of them such as angiotensin converting enzyme inhibitors, angiotensin II, and mineralocorticoid receptor inhibitors are already available and in clinical use (Table 1).

Mitochondrial production of ROS can be targeted through compounds such as MitoQ, a redox active mitochondrial targeted quinone [124], and others [125] that have been shown to be beneficial in both animal models as well as ongoing clinical trials. Additional studies are, however, needed to clarify the signaling pathways involved and long-term effects. NADPH oxidase induced production of ROS is another possible target [121], and its inhibition through the use of apocynin has already been shown to be effective against renal CaOx crystal deposition in rat models. Natural phenolic compounds have been shown to reduce oxidative stress in hypertension through inhibiting NADPH oxidase and scavenging free radicals [126]. Recent studies have shown involvement of NLRP3 inflammasome in crystal induced inflammation. Studies have also shown inhibition of this inflammasome leads to reduced inflammatory response and CaOx crystal deposition in rat model [89]. OLT1177, a B-sulfonyl nitrile compound, an inhibitor of NLRP3 is safe for human use [127].

Experimental as well as clinical investigations indicate a role for macrophage polarization in crystal deposition in the kidneys. Macrophage polarization has also been suggested to be involved in many diseases including atherosclerosis. M1 macrophages wield anti-inflammatory effect and promote the development of plaques. M2 macrophages, on the other hand, produce anti-inflammatory effects and reduce plaque formation [128]. Can this be true also in the development of interstitial RP? Could growth of RP be controlled through regulation of macrophage polarization [1]? Perhaps even through control of ROS [129]?

Our current understanding of the pathogenesis of idiopathic CaOx kidney stones points to two basic approaches for therapeutic intervention to reduce their recurrence: the reduction of supersaturation (which is the current clinical standard of care), and the reduction of inflammation. Inflammation is caused by the production of ROS and development of oxidative stress. Although clinical studies are limited in this area, a wide expanse of experimental data support that treatment of inflammation could be considered, along with standard evidence-based medications, when patients are being counselled for stone prevention.

Table 1 Prescription drugs or supplements that may benefit kidney stone formers.

Drug name	Mechanism of action	Indication	Potential use/benefit in stone formers
Atorvastatin	-HMG-CoA reductase competitive inhibitor	-Elevated serum total cholesterol, LDL cholesterol, or high triglycerides	-Reduced oxidative stress and CaOx crystal deposition by inhibiting NLRP3 inflammasome
Losartan	-Angiotensin II type 1 receptor antagonist	-Hypertension, reduce stroke risk, or diabetic nephropathy	-Reduced oxidative stress, anti-inflammatory effect by blocking angiotensin-1 receptor
Apocynin	-Selective inhibitor of phagocyte NADPH oxidase	-Plant-based, dietary supplement with few publications in humans	-Inhibition of ROS generation by NADPH oxidases and ROS scavenging
Lisinopril	-ACE inhibitor	-Acute myocardial infarction, heart failure, or hypertension	-Reduced expression of pro-inflammatory cytokines and attenuated cell activation (in particular macrophages)
Cyclosporine A	-Calcineurin inhibition, impaired T-cell activity	-Solid organ rejection, prophylaxis or rejection, or autoimmune diseases	-Selective inhibition of T-cell proliferation and macrophage-mediated accumulation in kidney
Spironolactone	-Mineralocorticoid receptor antagonist and diuretic	-Hypertension, or heart failure	-Reduced oxidative stress and reduced expression of pro-inflammatory cytokines

HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; NADPH, nicotinamide adenine dinucleotide phosphate; ACE, angiotensin converting enzyme; LDL, low-density lipoprotein; CaOx, calcium oxalate; NLRP3, pyrin domain containing-3; ROS, reactive oxygen species.
Note: none of these are approved by the US Food and Drug Administration for kidney stones or have been tested in humans for indication of kidney stone prevention.

Author contributions

Study concept and design: Saeed R. Khan.
Data acquisition: Saeed R. Khan, Benjamin K. Canales.
Data analysis: Saeed R. Khan, Benjamin K. Canales.
Drafting of manuscript: Saeed R. Khan, Benjamin K. Canales.
Critical revision of the manuscript: Saeed R. Khan, Benjamin K. Canales.

Conflicts of interest

The authors declare no conflict of interest.

References

[1] Khan SR, Canales BK, Dominguez-Gutierrez PR. Randall's plaque and calcium oxalate stone formation: role for immunity and inflammation. Nat Rev Nephrol 2021;17:417—33.
[2] Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, et al. Kidney stones. Nat Rev Dis Prim 2016;2:16008. https://doi.org/10.1038/nrdp.2016.8.
[3] Eisner BH, Goldfarb DS. A nomogram for the prediction of kidney stone recurrence. J Am Soc Nephrol 2014;25:2685—7.
[4] Daudon M, Jungers P. Diabetes and nephrolithiasis. Curr Diabetes Rep 2007;7:443—8.
[5] Obligado SH, Goldfarb DS. The association of nephrolithiasis with hypertension and obesity: a review. Am J Hypertens 2008;21:257—64.
[6] West B, Luke A, Durazo-Arvizu RA, Cao G, Shoham D, Kramer H. Metabolic syndrome and self-reported history of kidney stones: the National Health and Nutrition examination Survey (NHANES III) 1988—1994. Am J Kidney Dis 2008;51:741—7.
[7] Shoag J, Halpern J, Goldfarb DS, Eisner BH. Risk of chronic and end stage kidney disease in patients with nephrolithiasis. J Urol 2014;192:1440—5.
[8] Finlayson B. Physicochemical aspects of urolithiasis. Kidney Int 1978;13:344—60.
[9] Kok DJ, Khan SR. Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int 1994;46:847—54.
[10] Khan SR, Kok DJ. Modulators of urinary stone formation. Front Biosci 2004;9:1450—82.
[11] Finlayson B, Reid F. The expectation of free and fixed particles in urinary stone disease. Invest Urol 1978;15:442—8.
[12] Rodgers AL. Physicochemical mechanisms of stone formation. Urolithiasis 2017;45:27—32.
[13] Rodgers AL. Urinary saturation: casual or causal risk factor in urolithiasis? BJU Int 2014;114:104—10.
[14] Evan A, Lingeman J, Coe FL, Worcester E. Randall's plaque: pathogenesis and role in calcium oxalate nephrolithiasis. Kidney Int 2006;69:1313—8.
[15] Williams Jr JC, Al-Awadi H, Muthenini M, Bledsoe SB, El-Achkar T, Evan AP, et al. Stone morphology distinguishes two pathways of idiopathic calcium oxalate stone pathogenesis. J Endourol 2022;36:694—702.
[16] Khan SR, Rodriguez DE, Gower LB, Monga M. Association of Randall plaque with collagen fibers and membrane vesicles. J Urol 2012;187:1094—100.
[17] Evan AP, Bledsoe S, Worcester EM, Coe FL, Lingeman JE, Bergsland KJ. Renal inter-alpha-trypsin inhibitor heavy chain 3 increases in calcium oxalate stone-forming patients. Kidney Int 2007;72:1503—11.
[18] Carpentier X, Bazin D, Combes C, Mazouyes A, Rouzière S, Albouy PA, et al. High Zn content of Randall's plaque: a μ-X-ray fluorescence investigation. J Trace Elem Med Biol 2011;25:160—5.
[19] Khan SR, Finlayson B, Hackett R. Renal papillary changes in patient with calcium oxalate lithiasis. Urology 1984;23:194—9.
[20] Taguchi K, Hamamoto S, Okada A, Unno R, Kamisawa H, Naiki T, et al. Genome-wide gene expression profiling of Randall's plaques in calcium oxalate stone formers. J Am Soc Nephrol 2017;28:333—47.
[21] Taguchi K, Okada A, Hamamoto S, Unno R, Moritoki Y, Ando R, et al. M1/M2-macrophage phenotypes regulate renal calcium oxalate crystal development. Sci Rep 2016;6:35167. https://doi.org/10.1038/srep35167.
[22] Sun AY, Hinck B, Cohen BR, Keslar K, Fairchild RL, Monga M. Inflammatory cytokines in the papillary tips and urine of nephrolithiasis patients. J Endourol 2018;32:236—44.
[23] Coe FL, Evan AP, Lingeman JE, Worcester EM. Plaque and deposits in nine human stone diseases. Urol Res 2010;38:239—47.
[24] Huguet L, Le Dudal M, Livrozet M, Bazin D, Frochot V, Perez J, et al. High frequency and wide range of human kidney papillary crystalline plugs. Urolithiasis 2018;46:333—41.
[25] Khan SR, Atmani F, Glenton P, Hou Z, Talham DR, Khurshid M. Lipids and membranes in the organic matrix of urinary calcific crystals and stones. Calcif Tissue Int 1996;59:357—65.
[26] Witzmann FA, Evan AP, Coe FL, Worcester EM, Lingeman JE, Williams JC Jr. Label-free proteomic methodology for the analysis of human kidney stone matrix composition. Proteome Sci 2016;14:4. https://doi.org/10.1186/s12953-016-0093-x.
[27] Canales BK, Anderson L, Higgins L, Ensrud-Bowlin K, Roberts KP, Wu B, et al. Proteome of human calcium kidney stones. Urology 2010;76:1017.e13—20. https://doi.org/10.1016/j.urology.2010.05.005.
[28] Merchant ML, Cummins TD, Wilkey DW, Salyer SA, Powell DW, Klein JB, et al. Proteomic analysis of renal calculi indicates an important role for inflammatory processes in calcium stone formation. Am J Physiol Ren Physiol 2008;295:F1254—8. https://doi.org/10.1152/ajprenal.00134.2008.
[29] Okumura N, Tsujihata M, Momohara C, Yoshioka I, Suto K, Nonomura N, et al. Diversity in protein profiles of individual calcium oxalate kidney stones. PLoS One 2013;8:e68624. https://doi.org/10.1371/journal.pone.0068624.
[30] Boonla C, Tosukhowong P, Spittau B, Schlosser A, Pimratana C, Krieglstein K. Inflammatory and fibrotic proteins proteomically identified as key protein constituents in urine and stone matrix of patients with kidney calculi. Clin Chim Acta 2014;429:81—9.
[31] Khan SR, Hackett RL. Role of organic matrix in urinary stone formation: an ultrastructural study of crystal matrix interface of calcium oxalate monohydrate stones. J Urol 1993;150:239—45.
[32] Khan SR, Shevock PN, Hackett RL. Presence of lipids in urinary stones: results of preliminary studies. Calcif Tissue Int 1988;42:91—6.
[33] McKee MD, Nanci A, Khan SR. Ultrastructural immunodetection of osteopontin and osteocalcin as major matrix components of renal calculi. J Bone Miner Res 1995;10:1913—29.
[34] Romero V, Akpinar H, Assimos DG. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. Rev Urol 2010;12:e86—96. PMCID: PMC2931286.
[35] Scales CD Jr, Curtis LH, Norris RD, Springhart WP, Sur RL, Schulman KA, et al. Changing gender prevalence of stone disease. J Urol 2007;177:979—82.

[36] Siener R, Herwig H, Rudy J, Schaefer RM, Lossin P, Hesse A. Urinary stone composition in Germany: results from 45 783 stone analyses. World J Urol 2022;40:1813—20.
[37] Dinan MA, Robinson TJ, Zagar TM, Scales CD Jr, Curtis LH, Reed SD, et al. Changes in initial treatment for prostate cancer among Medicare beneficiaries, 1999—2007. Int J Radiat Oncol Biol Phys 2012;82:e781—6. https://doi.org/10.1016/j.ijrobp.2011.11.024.
[38] Rodgers A, Allie-Hamdulay S, Pinnock D, Baretta G, Trinchieri A. Risk factors for renal calcium stone formation in South African and European young adults. Arch Ital Urol Androl 2009;81:171—4.
[39] Rodgers AL. Race, ethnicity and urolithiasis: a critical review. Urolithiasis 2013;41:99—103.
[40] Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC. Time trends in reported prevalence of kidney stones in the United States: 1976—1994. Kidney Int 2003;63:1817—23.
[41] Strazzullo P, Barba G, Vuotto P, Farinaro E, Siani A, Nunziata V, et al. Past history of nephrolithiasis and incidence of hypertension in men: a reappraisal based on the results of the Olivetti Prospective Heart Study. Nephrol Dial Transplant 2001;16:2232—5.
[42] Beck BB, Baasner A, Buescher A, Habbig S, Reintjes N, Kemper MJ, et al. Novel findings in patients with primary hyperoxaluria type III and implications for advanced molecular testing strategies. Eur J Hum Genet 2013;21:162—72.
[43] Hsi RS, Spieker AJ, Stoller ML, Jacobs Jr DR, Reiner AP, McClelland RL, et al. Coronary artery calcium score and association with recurrent nephrolithiasis: the multi-ethnic study of atherosclerosis. J Urol 2016;195:971—6.
[44] Liu Y, Li S, Zeng Z, Wang J, Xie L, Li T, et al. Kidney stones and cardiovascular risk: a meta-analysis of cohort studies. Am J Kidney Dis 2014;64:402—10.
[45] Ferraro PM, Taylor EN, Eisner BH, Gambaro G, Rimm EB, Mukamal KJ, et al. History of kidney stones and the risk of coronary heart disease. JAMA 2013;310:408—15.
[46] Siener R. Nutrition and kidney stone disease. Nutrients 2021;13:1917. https://doi.org/10.3390/nu13061917.
[47] Ferraro PM, Cunha TDS, Taylor EN, Curhan GC. Temporal trends of dietary risk factors after a diagnosis of kidney stones. Clin J Am Soc Nephrol 2022;17:83—9.
[48] Crivelli JJ, Mitchell T, Knight J, Wood KD, Assimos DG, Holmes RP, et al. Contribution of dietary oxalate and oxalate precursors to urinary oxalate excretion. Nutrients 2020;13:62. https://doi.org/10.3390/nu13010062.
[49] Ticinesi A, Nouvenne A, Chiussi G, Castaldo G, Guerra A, Meschi T. Calcium oxalate nephrolithiasis and gut microbiota: not just a gut-kidney axis. A nutritional perspective. Nutrients 2020;12:548. https://doi.org/10.3390/nu12020548.
[50] Allison MJ, Cook HM, Milne DB, Gallagher S, Clayman RV. Oxalate degradation by gastrointestinal bacteria from humans. J Nutr 1986;116:455—60.
[51] Sidhu H, Schmidt ME, Cornelius JG, Thamilselvan S, Khan SR, Hesse A, et al. Direct correlation between hyperoxaluria/oxalate stone disease and the absence of the gastrointestinal tract-dwelling bacterium Oxalobacter formigenes: possible prevention by gut recolonization or enzyme replacement therapy. J Am Soc Nephrol 1999;10(Suppl. 14):S334—40. PMID: 10541258.
[52] Tavasoli S, Alebouyeh M, Naji M, Majd GS, Nashtaei MS, Broumandnia N, et al. Association of intestinal oxalate-degrading bacteria with recurrent calcium kidney stone formation and hyperoxaluria: a case-control study. BJU Int 2020;125:133—43.
[53] Miller AW, Choy D, Penniston KL, Lange D. Inhibition of urinary stone disease by a multi-species bacterial network ensures healthy oxalate homeostasis. Kidney Int 2019;96:180—8.
[54] Milliner D, Hoppe B, Groothoff J. A randomised Phase II/III study to evaluate the efficacy and safety of orally administered Oxalobacter formigenes to treat primary hyperoxaluria. Urolithiasis 2018;46:313—23.
[55] Miller AW, Penniston KL, Fitzpatrick K, Agudelo J, Tasian G, Lange D. Mechanisms of the intestinal and urinary microbiome in kidney stone disease. Nat Rev Urol 2022;19:695—707.
[56] Kok DJ, Iestra JA, Doorenbos CJ, Papapoulos SE. The effects of dietary excesses in animal protein and in sodium on the composition and the crystallization kinetics of calcium oxalate monohydrate in urines of healthy men. J Clin Endocrinol Metab 1990;71:861—7.
[57] Reddy ST, Wang CY, Sakhaee K, Brinkley L, Pak CY. Effect of low-carbohydrate high-protein diets on acid-base balance, stone-forming propensity, and calcium metabolism. Am J Kidney Dis 2002;40:265—74.
[58] Giannini S, Nobile M, Sartori L, Carbonare DL, Ciuffreda M, Corrò P, et al. Acute effects of moderate dietary protein restriction in patients with idiopathic hypercalciuria and calcium nephrolithiasis. Am J Clin Nutr 1999;69:267—71.
[59] Nouvenne A, Meschi T, Prati B, Guerra A, Allegri F, Vezzoli G, et al. Effects of a low-salt diet on idiopathic hypercalciuria in calcium-oxalate stone formers: a 3-mo randomized controlled trial. Am J Clin Nutr 2010;91:565—70.
[60] Taylor EN, Curhan GC. Dietary calcium from dairy and nondairy sources, and risk of symptomatic kidney stones. J Urol 2013;190:1255—9.
[61] Lopes HF, Martin KL, Nashar K, Morrow JD, Goodfriend TL, Egan BM. DASH diet lowers blood pressure and lipid-induced oxidative stress in obesity. Hypertension 2003;41:422—30.
[62] Taylor EN, Fung TT, Curhan GC. DASH-style diet associates with reduced risk for kidney stones. J Am Soc Nephrol 2009;20:2253—9.
[63] Holoch PA, Tracy CR. Antioxidants and self-reported history of kidney stones: the National Health and Nutrition Examination Survey. J Endourol 2011;25:1903—8.
[64] Shioi A, Ikari Y. Plaque calcification during atherosclerosis progression and regression. J Atherosclerosis Thromb 2018;25:294—303.
[65] Patel M, Yarlagadda V, Adedoyin O, Saini V, Assimos DG, Holmes RP, et al. Oxalate induces mitochondrial dysfunction and disrupts redox homeostasis in a human monocyte derived cell line. Redox Biol 2018;15:207—15.
[66] Niimi K, Yasui T, Hirose M, Hamamoto S, Itoh Y, Okada A, et al. Mitochondrial permeability transition pore opening induces the initial process of renal calcium crystallization. Free Radic Biol Med 2012;52:1207—17.
[67] Cohen AJ, Adamsky MA, Nottingham CU, Pruitt J, Lapin B, Wang CH, et al. Impact of statin intake on kidney stone formation. Urology 2019;124:57—61.
[68] Peng Y, Fang Z, Liu M, Wang Z, Li L, Ming S, et al. Testosterone induces renal tubular epithelial cell death through the HIF-1α/BNIP3 pathway. J Transl Med 2019;17:62. https://doi.org/10.1186/s12967-019-1821-7.
[69] Huang F, Li Y, Cui Y, Zhu Z, Chen J, Zeng F, et al. Relationship between serum testosterone levels and kidney stones prevalence in men. Front Endocrinol 2022;13:863675. https://doi.org/10.3389/fendo.2022.863675.
[70] Shakhssalim N, Gilani KR, Parvin M, Torbati PM, Kashi AH, Azadvari M, et al. An assessment of parathyroid hormone, calcitonin, 1,25 $(OH)_2$ vitamin D3, estradiol and testosterone in men with active calcium stone disease and evaluation of its biochemical risk factors. Urol Res 2011;39:1—7.

[71] Li JY, Zhou T, Gao X, Xu C, Sun Y, Peng Y, et al. Testosterone and androgen receptor in human nephrolithiasis. J Urol 2010; 184:2360—3.
[72] Basiri A, Naji M, Houshmand M, Shakhssalim N, Golestan B, Azadvari M, et al. CAG repeats and one polymorphism in androgen receptor gene are associated with renal calcium stone disease. Urologia 2022;89:391—6.
[73] Galán-Llopis JA, Sánchez-Pellicer P, Navarro-López V. Role of microbiome in kidney stone disease. Curr Opin Urol 2023;33: 84—9.
[74] Xie J, Huang JS, Huang XJ, Peng JM, Yu Z, Yuan YQ, et al. Profiling the urinary microbiome in men with calcium-based kidney stones. BMC Microbiol 2020;20:41. https://doi.org/10.1186/s12866-020-01734-6.
[75] Kachroo N, Monga M, Miller AW. Comparative functional analysis of the urinary tract microbiome for individuals with or without calcium oxalate calculi. Urolithiasis 2022;50: 303—17.
[76] Gao H, Lin J, Xiong F, Yu Z, Pan S, Huang Y. Urinary microbial and metabolomic profiles in kidney stone disease. Front Cell Infect Microbiol 2022;12:953392. https://doi.org/10.3389/fcimb.2022.953392.
[77] Khan SR. Nephrocalcinosis in animal models with and without stones. Urol Res 2010;38:429—38.
[78] Khan SR. Animal models of kidney stone formation: an analysis. World J Urol 1997;15:236—43.
[79] Khan SR, Glenton PA, Byer KJ. Modeling of hyperoxaluric calcium oxalate nephrolithiasis: experimental induction of hyperoxaluria by hydroxy-L-proline. Kidney Int 2006;70: 914—23.
[80] Zuo J, Khan A, Glenton PA, Khan SR. Effect of NADPH oxidase inhibition on the expression of kidney injury molecule and calcium oxalate crystal deposition in hydroxy-L-proline-induced hyperoxaluria in the male Sprague-Dawley rats. Nephrol Dial Transplant 2011;26:1785—96.
[81] Thamilselvan S, Menon M. Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. BJU Int 2005;96:117—26.
[82] Huang HS, Ma MC, Chen J, Chen CF. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. J Urol 2002;167:2584—93.
[83] Umekawa T, Hatanaka Y, Kurita T, Khan SR. Effect of angiotensin II receptor blockage on osteopontin expression and calcium oxalate crystal deposition in rat kidneys. J Am Soc Nephrol 2004;15:635—44.
[84] Tsujihata M, Yoshioka I, Tsujimura A, Nonomura N, Okuyama A. Why does atorvastatin inhibit renal crystal retention? Urol Res 2011;39:379—83.
[85] Martinon F. Signaling by ROS drives inflammasome activation. Eur J Immunol 2010;40:616—9.
[86] Liu Y, Sun Y, Kang J, He Z, Liu Q, Wu J, et al. Role of ROS-induced NLRP3 inflammasome activation in the formation of calcium oxalate nephrolithiasis. Front Immunol 2022;13:818625. https://doi.org/10.3389/fimmu.2022.818625.
[87] Mulay SR, Kulkarni OP, Rupanagudi KV, Migliorini A, Darisipudi MN, Vilaysane A, et al. Calcium oxalate crystals induce renal inflammation by NLRP3-mediated IL-1beta secretion. J Clin Invest 2013;123:236—46.
[88] Knauf F, Asplin JR, Granja I, Schmidt IM, Moeckel GW, David RJ, et al. NALP3-mediated inflammation is a principal cause of progressive renal failure in oxalate nephropathy. Kidney Int 2013;84:895—901.
[89] Joshi S, Wang W, Peck AB, Khan SR. Activation of the NLRP3 inflammasome in association with calcium oxalate crystal induced reactive oxygen species in kidneys. J Urol 2015;193: 1684—91.
[90] Sun Y, Liu Y, Guan X, Kang J, Wang X, Liu Q, et al. Atorvastatin inhibits renal inflammatory response induced by calcium oxalate crystals via inhibiting the activation of TLR4/NF-kappaB and NLRP3 inflammasome. IUBMB Life 2020;72:1065—74.
[91] Liang L, Li L, Tian J, Lee SO, Dang Q, Huang CK, et al. Androgen receptor enhances kidney stone-CaOx crystal formation via modulation of oxalate biosynthesis & oxidative stress. Mol Endocrinol 2014;28:1291—303.
[92] Zhu W, Zhao Z, Chou F, Zuo L, Liu T, Yeh S, et al. Loss of the androgen receptor suppresses intrarenal calcium oxalate crystals deposition via altering macrophage recruitment/M2 polarization with change of the miR-185-5p/CSF-1 signals. Cell Death Dis 2019;10:275. https://doi.org/10.1038/s41419-019-1358-y.
[93] Anders HJ, Suarez-Alvarez B, Grigorescu M, Foresto-Neto O, Steiger S, Desai J, et al. The macrophage phenotype and inflammasome component NLRP3 contributes to nephrocalcinosis-related chronic kidney disease independent from IL-1-mediated tissue injury. Kidney Int 2018;93:656—69.
[94] Letavernier E, Kauffenstein G, Huguet L, Navasiolava N, Bouderlique E, Tang E, et al. ABCC6 deficiency promotes development of Randall plaque. J Am Soc Nephrol 2018;29: 2337—47.
[95] Grewal JS, Tsai JY, Khan SR. Oxalate-inducible AMBP gene and its regulatory mechanism in renal tubular epithelial cells. Biochem J 2005;387:609—16.
[96] Okada A, Yasui T, Fujii Y, Niimi K, Hamamoto S, Hirose M, et al. Renal macrophage migration and crystal phagocytosis via inflammatory-related gene expression during kidney stone formation and elimination in mice: detection by association analysis of stone-related gene expression and microstructural observation. J Bone Miner Res 2010;25: 2701—11.
[97] Khan SR. Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissue-culture studies. Clin Exp Nephrol 2004;8:75—88.
[98] Khan SR, Joshi S, Wang W, Peck AB. Regulation of macromolecular modulators of urinary stone formation by reactive oxygen species: transcriptional study in an animal model of hyperoxaluria. Am J Physiol Ren Physiol 2014;306:F1285—95. https://doi.org/10.1152/ajprenal.00057.2014.
[99] Joshi S, Clapp WL, Wang W, Khan SR. Osteogenic changes in kidneys of hyperoxaluric rats. Biochim Biophys Acta 2015; 1852:2000—12.
[100] Mo L, Liaw L, Evan AP, Sommer AJ, Lieske JC, Wu XR. Renal calcinosis and stone formation in mice lacking osteopontin, Tamm-Horsfall protein, or both. Am J Physiol Ren Physiol 2007;293:F1935—43. https://doi.org/10.1152/ajprenal.00383.2007.
[101] Liu Y, Mo L, Goldfarb DS, Evan AP, Liang F, Khan SR, et al. Progressive renal papillary calcification and ureteral stone formation in mice deficient for Tamm-Horsfall protein. Am J Physiol Ren Physiol 2010;299:F469—78. https://doi.org/10.1152/ajprenal.00243.2010.
[102] Umekawa T, Byer K, Uemura H, Khan SR. Diphenyleneiodium (DPI) reduces oxalate ion- and calcium oxalate monohydrate and brushite crystal-induced upregulation of MCP-1 in NRK 52E cells. Nephrol Dial Transplant 2005;20: 870—8.
[103] Gaspar S, Niculite C, Cucu D, Marcu I. Effect of calcium oxalate on renal cells as revealed by real-time measurement of extracellular oxidative burst. Biosens Bioelectron 2010;25: 1729—34.
[104] Khan SR. Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. Urol Res 2005;33:349—57.
[105] Meimaridou E, Jacobson J, Seddon AM, Noronha-Dutra AA, Robertson WG, Hothersall JS. Crystal and microparticle

effects on MDCK cell superoxide production: oxalate-specific mitochondrial membrane potential changes. Free Radic Biol Med 2005;38:1553—64.
[106] Khan SR, Khan A, Byer KJ. Temporal changes in the expression of mRNA of NADPH oxidase subunits in renal epithelial cells exposed to oxalate or calcium oxalate crystals. Nephrol Dial Transplant 2011;26:1778—85.
[107] Huang HS, Chen J, Chen CF, Ma MC. Vitamin E attenuates crystal formation in rat kidneys: roles of renal tubular cell death and crystallization inhibitors. Kidney Int 2006;70:699—710.
[108] Habibzadegah-Tari P, Byer KG, Khan SR. Reactive oxygen species mediated calcium oxalate crystal-induced expression of MCP-1 in HK-2 cells. Urol Res 2006;34:26—36.
[109] Thamilselvan V, Menon M, Thamilselvan S. Oxalate-induced activation of PKC-alpha and -delta regulates NADPH oxidase-mediated oxidative injury in renal tubular epithelial cells. Am J Physiol Ren Physiol 2009;297:F1399—410. https://doi.org/10.1152/ajprenal.00051.2009.
[110] Cao LC, Honeyman TW, Cooney R, Kennington L, Scheid CR, Jonassen JA. Mitochondrial dysfunction is a primary event in renal cell oxalate toxicity. Kidney Int 2004;66:1890—900.
[111] Farooq SM, Boppana NB, Devarajan A, Sekaran SD, Shankar EM, Li C, et al. C-phycocyanin confers protection against oxalate-mediated oxidative stress and mitochondrial dysfunctions in MDCK cells. PLoS One 2014;9:e93056. https://doi.org/10.1371/journal.pone.0093056.
[112] Zhang J, Wang Q, Xu C, Lu Y, Hu H, Qin B, et al. MitoTEMPO prevents oxalate induced injury in NRK-52E cells via inhibiting mitochondrial dysfunction and modulating oxidative stress. Oxid Med Cell Longev 2017;2017:7528090. https://doi.org/10.1155/2017/7528090.
[113] Kusmartsev S, Dominguez-Gutierrez PR, Canales BK, Bird VG, Vieweg J, Khan SR. Calcium oxalate stone fragment and crystal phagocytosis by human macrophages. J Urol 2016;195:1143—51.
[114] Xi J, Chen Y, Jing J, Zhang Y, Liang C, Hao Z, et al. Sirtuin 3 suppresses the formation of renal calcium oxalate crystals through promoting M2 polarization of macrophages. J Cell Physiol 2019;234:11463—73.
[115] Xi J, Jing J, Zhang Y, Liang C, Hao Z, Zhang L, et al. SIRT3 inhibited the formation of calcium oxalate-induced kidney stones through regulating NRF2/HO-1 signaling pathway. J Cell Biochem 2019;120:8259—71.
[116] Khan SR. Is oxidative stress, a link between nephrolithiasis and obesity, hypertension, diabetes, chronic kidney disease, metabolic syndrome? Urol Res 2012;40:95—112.
[117] Chinetti-Gbaguidi G, Daoudi M, Rosa M, Vinod M, Louvet L, Copin C, et al. Human alternative macrophages populate calcified areas of atherosclerotic lesions and display impaired RANKL-induced osteoclastic bone resorption activity. Circ Res 2017;121:19—30.
[118] Newby AC. Metalloproteinases promote plaque rupture and myocardial infarction: a persuasive concept waiting for clinical translation. Matrix Biol 2015;44—6:157—66.
[119] Wu Y, Zhang J, Li C, Hu H, Qin B, Wang T, et al. The activation of ROS/NF-kappaB/MMP-9 pathway promotes calcium-induced kidney crystal deposition. Oxid Med Cell Longev 2021;2021:8836355. https://doi.org/10.1155/2021/8836355.
[120] Newby AC. Proteinases and plaque rupture: unblocking the road to translation. Curr Opin Lipidol 2014;25:358—66.
[121] Joshi S, Peck AB, Khan SR. NADPH oxidase as a therapeutic target for oxalate induced injury in kidneys. Oxid Med Cell Longev 2013;2013:462361. https://doi.org/10.1155/2013/46361.
[122] Zahid A, Li B, Kombe AJK, Jin T, Tao J. Pharmacological inhibitors of the NLRP3 inflammasome. Front Immunol 2019;10:2538. https://doi.org/10.3389/fimmu.2019.02538.
[123] Garrelfs SF, Frishberg Y, Hulton SA, Koren MJ, O'Riordan WD, Cochat P, et al. Lumasiran, an RNAi therapeutic for primary hyperoxaluria type 1. N Engl J Med 2021;384:1216—26.
[124] Zinovkin RA, Zamyatnin AA. Mitochondria-targeted drugs. Curr Mol Pharmacol 2019;12:202—14.
[125] Daenen K, Andries A, Mekahli D, Van Schepdael A, Jouret F, Bammens B. Oxidative stress in chronic kidney disease. Pediatr Nephrol 2019;34:975—91.
[126] Yousefian M, Shakour N, Hosseinzadeh H, Hayes AW, Hadizadeh F, Karimi G. The natural phenolic compounds as modulators of NADPH oxidases in hypertension. Phytomedicine 2019;55:200—13.
[127] Marchetti C, Swartzwelter B, Gamboni F, Neff CP, Richter K, Azam T, et al. OLT1177, a beta-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation. Proc Natl Acad Sci U S A 2018;115:E1530—9. https://doi.org/10.1073/pnas.1716095115.
[128] Yang S, Yuan HQ, Hao YM, Ren Z, Qu SL, Liu LS, et al. Macrophage polarization in atherosclerosis. Clin Chim Acta 2020;501:142—6.
[129] Tan HY, Wang N, Li S, Hong M, Wang X, Feng Y. The reactive oxygen species in macrophage polarization: reflecting its dual role in progression and treatment of human diseases. Oxid Med Cell Longev 2016;2016:2795090. https://doi.org/10.1155/2016/2795090.