WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 2, 413-421. Review Article ISSN 2277– 7105

ROLE OF MODULATORS IN VARIOUS PHASES OF CRYSTALLIZATION IN RENAL STONE FORMATION

Monika Gupta*

Associate Professor in Chemistry, Vaish College, Rohtak.

Article Received on
28 Nov. 2022,

Revised on 18 Dec. 2022,
Accepted on 08 Jan. 2023

DOI: 10.20959/wjpr20232-26932

***Corresponding Author**
Dr. Monika Gupta
Associate Professor in Chemistry, Vaish College, Rohtak.

ABSTRACT

Renal stones have badly affected human beings for millennia. It is the most universal chronic disease of urinary tract influencing about 10% of the total population. Many researchers are trying to reveal the method of formation of renal stones. Renal stone formation is a multifactorial problem & includes nucleation, crystal growth, aggregation and retention. Whatever, the basic cause of stone disease, nutritional, environmental or genetic factor, the actual formation of stone in kidney may be looked upon as a strictly physiochemical problem. This review paper accounts for a broad description of the process of renal stone formation & the role of modulators viz. inhibitors & promoters in different phases of renal stone crystallization.

KEYWORDS: Renal stone, Nucleation, Crystal growth, Aggregation & Retention, Modulators, Inhibitors, Promoters.

1. INTRODUCTION

Renal stones are polycrystalline aggregates comprising of various amounts of organic and inorganic matrix components. Different types of chemical disturbances in the urine produce stones with various chemical compositions and shapes. The most common urinary stone types are calcium oxalate, calcium phosphate, cystine, uric acid and struvite.[1]

Calcium Oxalate & Calcium Phosphate Stones are the most familiar type of renal stones. Around 85% of the urinary stones in human beings are calcium stones made up of oxalate and phosphate. Struvite stones account for only 5% of all renal stones. Struvite stones are generally caused by a urinary infection with bacteria producing the enzyme urease that neutralize urinary acid and cause them to grow. Cystine stones constitute only 1% of all

kidney stones. These are caused by over secretion of the amino acid cystine due to a genetic abnormality. Uric acid stones constitute about 10 % of kidney stones & are due to decreased ammonia excretion.[2]

The different types of renal stones along with their percentage occurrences are shown in Fig 1.

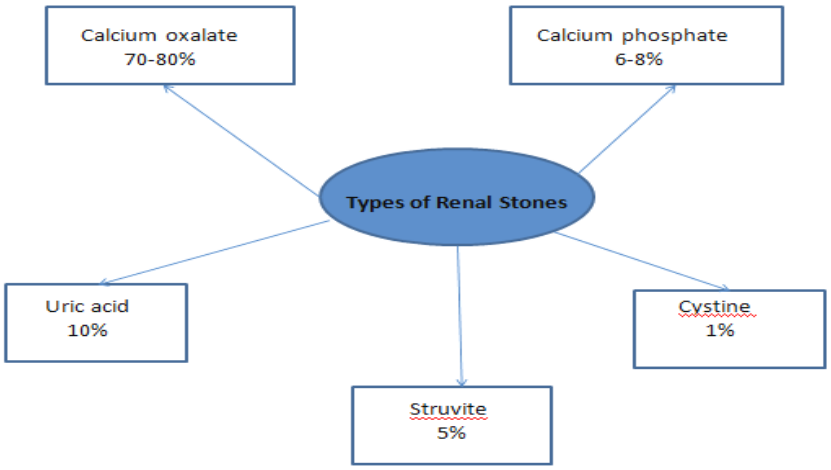

Figure 1: Different types of renal Stones and Their percentage occurrences.

The renal stone formation is eventually a multi-step process. The series of steps for renal stone formation is: Saturated urine → Super-saturation → Nucleation → Crystal growth→ Crystal Aggregation → Crystal Retention → Formation of renal stone. Calcium stone formation engages diverse phases of increasing accumulation of calcium phosphate (CaP) & calcium oxalate (CaOx) and includes – nucleation, followed by crystal growth, then crystal aggregation & finally crystal retention. Nucleation is the development of a solid crystal phase in the supersaturated urine. It is a crucial step in renal stone formation. Normal urine is generally super-saturated with CaOx. But when the urine become more concentrated, then solubility product (Ksp) exceeds the formation product (Kf) and spontaneous homogeneous nucleation will happen.[3]

It is found that calcium oxalate crystals in urine nucleate through a heterogeneous, not homogeneous, process. Homogeneous nucleation occurs without any external factors to initiate the process. On the other hand, heterogeneous nucleation requires the presence of nucleation-inducing species such as foreign particles, epithelial cells, cell remains & bacteria etc. As urine is intrinsically made up of numerous components in various concentrations (i.e. salts, metabolites and proteins), and heterogeneous nucleation requires less driving force to

occur than homogeneous nucleation, a heterogeneous origin of nucleation of calcium oxalate crystals is more probable.[4] When the Kf of heterogeneous nucleation is reached, crystal formation occurs. Crystallization corresponds to the initial phase of stone formation in urinary tract. Flowchart of the probable steps in renal stone formation is shown in Figure 2.

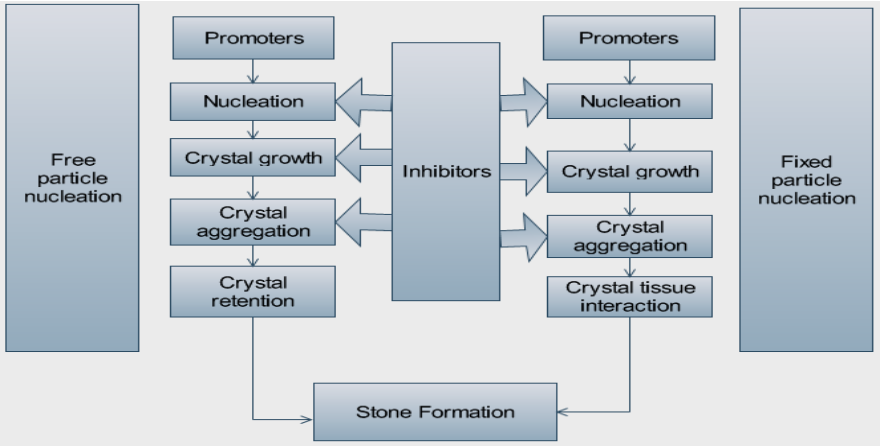

Figure 2: Flowchart of the probable steps in renal stone formation.

After the nucleation, now crystal growth is the subsequent most vital step of renal stone formation. The thrust for this process is a decrease in the P.E. of the atoms or molecules when they bind together. In a supersaturated solution, the free energy is enhanced by surface tension; however, this is considerable only when the size of cluster is less. Crystal growth is decided by the shape & size of the molecule, the physical composition of the matrix, super saturation levels & imperfections that exist in the structure of crystal.[5]

After crystal growth, crystal aggregation describes the process of binding the crystals together which results in the formation of large size clusters. Large aggregates are found more often in the urine of SF than in NSF. Aggregation of both CaOx & CaP crystals occurs easily when the solution is super-saturated. Aggregation depends on vander Waals forces, solid bridges & viscous binding that facilitate to hold the particles collectively, whereas disaggregation depends on electrostatic repulsion known as zeta potential. A less inter-particle distance raises the forces of attraction and favors crystal aggregation. In different steps of stone formation aggregation of crystals is the most important step rather than nucleation & growth as it occurs within fraction of seconds. Moreover, damage from free radicals also provides a proper surface for nucleation of calcium phosphate and oxalate.[6] This causes aggregation pursued by adherence of calcium phosphate aggregates to the renal tubules. Recently formed crystals now stick to the tubules & cause crystal retention and hence development of pathological renal stone.[7]

After crystal aggregation, crystals are now retained & get gathered in kidney. Crystal retention can be caused if the crystals grow outsized enough to be trapped in tubules or can stick to the renal tubular epithelial cells before excretion. Crystal formation generally depends on the composition of the fluid present in the renal tubules while their retention depends on the composition of the surface of renal tubular epithelial cell.[8] A non-adherent surface of the tubules and the urinary tract promotes retention of crystals. It was found that Glycosaminoglycans (GAGs), hyaluronic acid, osteopontin, & some other urinary macromolecular inhibitors have a vital responsibility in the progression of crystal retention.[9]

2. Modulators of renal stones

Urine contains a lot of ions or molecules out of which some can hinder or the others can support the process of renal stone formation. Inhibitors are the substances that can augment the super- saturation needed to start nucleation, lessen the growth & aggregation of crystals and reduce nucleation whereas promoters reduces the Kf of the super-saturated solution. The failure of equilibrium between the urinary inhibitors & promoters enhance the threat of stone formation.[10] The various parts of calcium oxalate crystallization are diagrammatically shown in Figure 3. Crystal nucleation followed by their growth, aggregation & retention are the essential steps in stone formation. Nucleation is presumed to be induced by one or more than a few substances known as promoters. Growth and aggregation of calcium oxalate crystals occur when formation product (Kf) exceeds the solubility product (Ksp). All these processes are neutralized by inhibitors.[11]

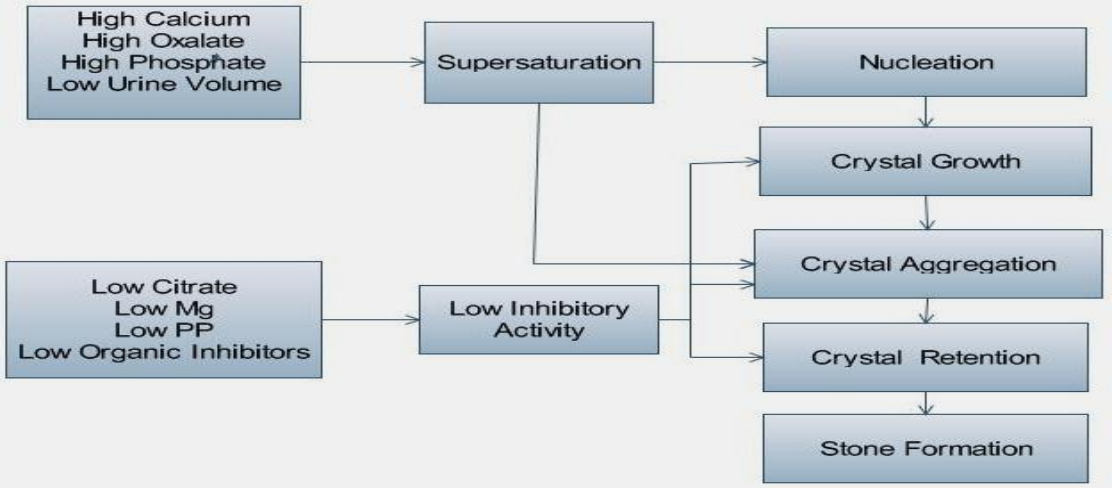

Figure 3: Major steps in calcium oxalate crystallization.

Generally, three types of modulators are known in our urine – one is low molecular weight compounds like magnesium, citrate & pyrophosphate; second ones are high molecular weight

proteins like Osteopontin (Uropontin), nephrocalcin, Tamm- Horsfall protein, UPTF1; third ones are lipids that are high Molecular weight non-protein compounds like mucopolysaccharides, GAGs etc. These alter the process of crystal formation & retention directly by interacting with the crystal or sometimes indirectly by effecting the composition of urine.[12-14]

2.1 Low molecular weight compounds

Pyrophosphate is a low molecular weight effective inhibitor of crystal growth. In some cases, it also results in crystal aggregation. At low concentrations approximately of the order of 16µM, PP inhibits CaOx crystal growth to about 50%. The pyrophosphate levels (20-40µM) in urine are sufficient to inhibit CaOX and CaP crystallisation. Citrate is another low molecular weight effective inhibitor of CaOx and CaP stones. Citric acid is present in blood that forms complexes with sodium, calcium and magnesium at pH levels of around 7.4. It was found that high protein diet reduces concentration of citrate levels in urine.[15] Moreover, lithium, magnesium, Parathormone (PTH), calcitonin & vitamin D increase the concentration of citrate levels in urine. Citrate reduces the concentration of Ca by forming complex with calcium thereby reducing formation of CaOx. Citrate is also found to increase the aggregation [16]. Magnesium have tendency to form complexes with oxalate, thereby reducing the oxalate required for supersaturation. It acts as a effective inhibitor of crystal growth. Stone formers are given oral supplements of Mg as it decreases oxalate absorption and secondly it increases the secretion of citrate in the urine.

Table 1: Role of low molecular weight compounds in various phases of crystallization of renal stones.

S. N.	Name of Modulator	Various Phases Of Crystallization			
		Nucleation	Crystal growth	Crystal aggregation	Crystal retention
1	Pyrophosphate	-	Inhibit	Inhibit	-
2	Citrate	-	Inhibit	-	-
3	Magnesium	-	Inhibit	-	-

2.2 High molecular weight protein compounds

Stone matrix is made up of a large number of proteins that affect one or another phase of crystallization. Some of them are nephrocalcin, osteopontin, Tamm-Horsfall protein, Renal lithostathine, UPTF1, albumin, collagen, bilkunin, hyaluronic acid (HL), nucleonin, inter-alpha-inhibitor and many more.[17-18] Tamm-Horsfall protein is the most abundant

glycoprotein found in the urine. The effect of various protein compounds on various phases of crystallization of stone formation is shown in Table 2.

Table 2: Role of high molecular weight protein compounds in various phases of crystallization of renal stones.

S. N.	Name of modulator	Various Phases Of Crystallization			
		Nucleation	Crystal growth	Crystal aggregation	Crystal retention
1	Nephrocalcin (NC)	Inhibit	Inhibit	Inhibit	-
2	Tamm-Horsfall protein(THP)	Promote	-	Promote /Inhibit	-
3	Osteopontin	Inhibit	Inhibit	Inhibit	Promote /Inhibit
4	Urinary Prothrombin fragment1(UPTF1)	Inhibit	Inhibit	Inhibit	
5	Albumin	Promote	-	Inhibit	-
6	Bikunin	Inhibit	Inhibit	Inhibit	Inhibit
7	Inter-alpha-inhibitor	Inhibit	Inhibit	Inhibit	Inhibit
8	Renal Lithostathine (RL)	-	Inhibit	-	-
9	α-Defensin	-	Promote	Promote	-
10	Nucleolin	-	-	-	Promote
11	Hyaluronic acid	-	-	-	Promote
12	Chondroitin sulphate	-	Inhibit	Inhibit	-
13	Heparin Suplphate	-	Inhibit	-	-
14	Annexin II	-	-	-	Promote
15	CD44	-	-	-	Promote
16	Histone HIB	-	Promote	-	-
17	Fibronectin	-	-	Inhibit	Inhibit
18	Collagen	Promote	-	-	-
19	Human urinary trefoil factor 1	-	Inhibit	-	-
20	Matrix Gla Protein (MGP)	-	Inhibit	-	Inhibit
21	Calgranulin	-	Inhibit	Inhibit	-

2.3 High molecular weight Non-protein compounds

The matrix of renal stones are composed of high Molecular weight non-protein compounds like glycolipids, phospholipids, mucopolysaccharides, GAGs etc. These can catalyze the nucleation of stones from the metastable urine present in renal tubules & play a vital role in renal stone formation.[19] Their effect on various phases of crystallization of stone formation is shown in Table 3.

Table 3: Role of high molecular weight non-protein compounds in various phases of crystallization of renal stones.

S. N.	Name of modulator	Various Phases Of Crystallization			
		Nucleation	Crystal growth	Crystal aggregation	Crystal retention
1	Glycosaminoglycans (GAGs)	-	Inhibit	Inhibit	Inhibit
2	Phosphatidylcholine (PC)	Promote	Promote	-	-
3	Cardiolipin(CL)	Promote	-	-	-
4	Lysophosphatidic acid (PA)	Promote	-	-	-

3. CONCLUSION

Renal stone formation occurs due to super-saturation of urine with some stone forming substances & finally their retention in the urinary tract. Stone formation is a multistep and very complex process and is affected by various compounds present in the stone matrix. A comprehensive discussion has been made about the various modulators and whether they promote or inhibit a particular phase of crystallization, thereby providing a better understanding of the pathogenesis of renal stones. Still many facets of progression of stone formation are not clear and require further research so that renal stone formation can be prevented.

4. REFERENCES

1. Coe FL, Favus MJ and Asplin JR .Nephrolithiasis. In Brenner & Rector's The Kidney Int, 7 ed.; Brenner BM and Ed. Saunders Elsevier: Philadelphia, 2004; 2: 1819 -1866.
2. Monika, Gupta, Bhayana Seema, and S. K. Sikka. "Pathophysiology of Calcium Containing Renal Stones." International Journal of Physical, Chemical and Mathematical Sciences, 2012; 1(1): 1-7.
3. Wang, Zhu, et al. "Recent advances on the mechanisms of kidney stone formation." International Journal of Molecular Medicine, 2021; 48(2): 1-10.
4. Viljoen, Adie, Rabia Chaudhry, and John Bycroft. "Renal stones." Annals of clinical biochemistry, 2019; 56 (1): 15-27.
5. Espinosa-Ortiz, Erika J., et al. "Current insights into the mechanisms and management of infection stones." Nature Reviews Urology, 2019; 16(1): 35-53.
6. Mager, R., and A. Neisius. "Current concepts on the pathogenesis of urinary stones." Der Urologe. Ausg. A, 2019; 58(11): 1272-1280.

7. Arafa, Ahmed, Ehab S. Eshak, and Hiroyasu Iso. "Oxalates, urinary stones and risk of cardiovascular diseases." Medical Hypotheses, 2020; 137: 109570.
8. Gupta, Shruti, and S. K. Shamsher. "Kidney Stones: Mecha-nism of Formation, Pathogenesis and Possible Treatments." J. Biomol. Biochem, 2018; 2: 1-5.
9. Wallace, Brendan, et al. "The Role of Urinary Modulators in the Development of Infectious Kidney Stones." Journal of Endourology, 2022. http://doi.org/10.1089/end.2022.0458
10. Gupta Monika, Bhayana Seema, Sikka SK. Role of urinary inhibitors and promoters in calcium oxalate crystallisation. International Journal of Research in Pharmacy and Chemistry, 2011; 1: 793-798.
11. Jappie, Dalielah, et al. "Seeking consistency for the role of urinary macromolecules and glycosaminoglycans in calcium oxalate crystallization processes pertaining to the risk of renal stone formation using a multi-faceted basic science approach." Clinica Chimica Acta, 2021; 521: 76-84.
12. Gupta, M. "A study to correlate urinary output and total organic acids of urine in calcium oxalate renal stone formers and controls". Journal of Advanced Scientific Research, 2019; 10(4): 2: 328-32, http://sciensage.info/index.php/JASR/article/view/394
13. Sassanarakkit, Supatcha, Paleerath Peerapen, and Visith Thongboonkerd. "StoneMod: a database for kidney stone modulatory proteins with experimental evidence." Scientific reports, 2020; 10(1): 1-9.
14. Peerapen, Paleerath, and Visith Thongboonkerd. "Kidney stone proteomics: an update and perspectives." Expert Review of Proteomics, 2021; 18(7): 557-569.
15. Seema, and Monika Gupta. "A study to evaluate the total organic acid content in urine of calcium oxalate renal stone formers and non stone formers." International journal of pharmaceutical sciences and research", 2018; 9(6): 2585-2588.
16. V. Kumar, S. Yu, G. Farell, F. G. Toback, and J. C. Lieske, "Renal epithelial cells constitutively produce a protein that blocks adhesion of crystals to their surface," American Journal of Physiology, 2004; 287(3): F373–F383.
17. C. F. Verkoelen, "Crystal retention in renal stone disease: a crucial role for the glycosaminoglycan hyaluronan?" Journal of the American Society of Nephrology, 2006; 17(6): 1673–1687.
18. S. Aggarwal, C. Tandon, M. Forouzandeh, S. K. Singla, R. Kiran, and R. K. Jethi, "Role of a protein inhibitor isolated from human renal stone matrix in urolithiasis," Indian Journal of Biochemistry and Biophysics, 2005; 42(2): 113–117.

19. Kanu Priya Aggarwal, Shifa Narula, Monica Kakkar, and Chanderdeep Tandon "Nephrolithiasis: Molecular Mechanism of Renal Stone Formation and the Critical Role Played by Modulators" BioMed Research International, 2013; 1-21.