INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

*Research Article*

# ROLE OF URINARY INHIBITORS AND PROMOTERS IN CALCIUM OXALATE CRYSTALLISATION

**Monika Gupta[1]*, Seema Bhayana[2] and S.K. Sikka[3]**

[1]Department of Chemistry, Vaish (P.G.) College, Rohtak, Haryana, India.
[2]UIET, M.D.University, Rohtak, Haryana, India.
[3]Vaish (P.G.) College, Rohtak, Haryana, India.

***Corresponding Author: guptamonika77@yahoo.com**

**ABSTRACT**

Urine of most people is supersaturated with stone forming constituents, including calcium, oxalate, phosphate and uric acid. Crystals or foreign bodies can act as nidi, upon which ions from the supersaturated urine form microscopic crystalline structures. The majority of urinary calculi contain calcium. Calcium stones occur when urine becomes supersaturated with calcium oxalate and phosphate. They form crystals that bind into hardened mineral deposits known as renal stones. The process of stone formation includes crystal nucleation, growth, aggregation and retention. Various substances in our urine modify these stone forming processes, thereby influencing a person's ability to promote or inhibit stone formation. Promoters of stone formation facilitate stone formation while inhibitors prevent it. Low urine volume, low urine pH, calcium, sodium, oxalate, and urate are known to promote stone formation. Many inorganic (eg. Citrate, magnesium, pyrophosphate etc.) and organic (eg. Tamm-Horsfall protein, glycosaminoglycans, uropontin, nephrocalcin, renal lithostathine etc.) Substances, high urine volume are known to inhibit stone formation. This paper presents role of urinary inhibitors and promoters in calcium oxalate crystallization.

**Keywords:** Renal stones, urinary calculi, promoters and inhibitors.

## 1. INTRODUCTION

Urine is a more complex solution, and is often supersaturated with respect to stone forming substances. But crystallization does not occur in urine because urine has the capacity to hold more solute than water as it contains a mixture of many electrically active ions that interact with each other, affecting their solubility. The presence of organic molecules, such as urea, uric acid and citrate, Tamm-Horsfall protein, glycosaminoglycans, uropontin, etc. affects the solubility of other substances.[1] Urine can hold large concentrations of solute above the solubility product, Ksp, and is therefore described as being 'metastable'. As the concentration of the substance is increased further, a point is reached when it can not be held in solution and get crystallizes and this concentration is known as the 'formation product' ($K_F$) of the substance. The formation product is the level of saturation at which solute no longer remains in solution and spontaneous nucleation occurs. The formation of urinary calculi requires a complex combination of factors; both promoting and inhibiting stone formation.[2] Coe et al found that inhibitory activity was low in urine of

stone formers as compared to normal subjects.[3]

## 2. INHIBITORS AND PROMOTERS

Inhibitors are the molecules which increase the supersaturation required to initiate nucleation, decrease the crystal growth and aggregation and inhibit secondary nucleation (deposition of new crystals on pre-existing crystal surfaces of similar type) whereas promoters causes reduction of the formation product of the supersaturated solution. The loss of balance between the urinary promoters and inhibitors has been suggested to increase the risk of stone formation more than disturbance in any single substance. The Table 1 give a brief idea about inhibitors and promoters of stone formation.

**Table 1: Inhibitors and Promoters of kidney stones**

| | Inhibitors | Promoters |
|---|---|---|
| **Inorganic-** | Citrate | Calcium |
| | Magnesium | Sodium |
| | Pyrophosphate | Oxalate |
| **Organic-** | Tamm-Horsfall protein (THP) | Urate |
| | Urinary Prothrombin fragment1 | Low urine pH |
| | Renal lithostathine | Tamm-Horsfall protein |
| | Glycosaminoglycans | |
| | Osteopontin(Uropontin) | |
| | Nephrocalcin | **Low urine volume** |
| | **High urine volume** | |

### 2.1 Inhibitors

Inhibitors slow or inhibit the rate of growth or aggregation of crystals or reduce adherence of crystals to the renal epithelium. Many inorganic and organic substances, high urine volume are known to inhibit crystal growth, aggregation and adhesion as shown in Table 2.

**Table 2: Effects of Inhibitors on Crystallization**

| Name of Inhibitor | Effect on Crystallization |
|---|---|
| Citrate | Inhibitor of growth |
| Magnesium | Inhibitor of growth |
| Pyrophosphate | Inhibitor of growth and aggregation |
| Osteopontin | Inhibitor of nucleation, growth and aggregation |
| Urinary Prothrombin fragment1 | Inhibitor of growth, aggregation and adhesion |
| Tamm-Horsfall protein (THP) | Promoter of nucleation and growth, inhibitor of aggregation |
| Glycosaminoglycans (GAGs) | Inhibitor of growth, aggregation and adhesion |
| Renal Lithostathine (RL) | Inhibitor of growth |
| Nephrocalcin (NC) | Inhibitor of growth and aggregation |

**2.1.1. Inorganic inhibitors** - Among inorganic inhibitors shown in Table 1, Citrate is the most important inhibitor. Magnesium may act indirectly by increasing urinary citrate levels. Pyrophosphate acts as inhibitor by reducing the absorption of calcium. These three inorganic inhibitors are discussed in the following sub-sections.

**2.1.1.1. Citrate**

Citric acid is a tricarboxylic acid that circulates in blood complexed to calcium, magnesium and sodium at physiological pH of 7.4. It is derived from both endogenous (Kreb's cycle) and exogenous sources (citrus fruits like orange and grapes). Any condition that results in intracellular acidosis, high protein diet and exercise will cause a decrease in urinary citrate concentrations. Parathormone (PTH),

magnesium, lithium, calcitonin, and vitamin D have been found to increase urinary citrate levels. Citrate is a potent inhibitor of calcium oxalate and phosphate stones in particular. Citrate seems to be an altering factor of both calcium oxalate monohydrate (thermodynamically most stable form) and calcium phosphate crystallization.[4] It forms complex with calcium thereby reducing the concentration of calcium oxalate. Citrate also increases the stone aggregation inhibitory activity of other urine macromolecules (e.g. THP) and may reduce the expression of urinary osteopontin, an important component of the protein matrix of urinary stones.[5] Moreover; urinary citrate excretion can increase urinary pH, a factor in the calcium-citrate-phosphate complex formation. Urinary alkalization of potassium citrate increases the solubility of uric acid thus preventing the salting out of calcium oxalate by urate.[6]

**2.1.1.2. Magnesium**

Magnesium is the fourth most abundant mineral in the body and is largely found in bones.

In a supersaturated calcium oxalate solution 2.0 mmol/L magnesium was shown to reduce crystal growth by 50%.[7] Magnesium can form complexes with oxalate thereby decreasing supersaturation. Oral intake of magnesium appears to decrease the oxalate absorption and urinary excretion, in a manner similar to calcium by binding to oxalate in the gut.[8] Subjects with magnesium deficiency after being provided with magnesium supplements showed increase of excretion of citrate in urine.[9]

**2.1.1.3. Pyrophosphate**

Pyrophosphate is a potent inhibitor of crystal growth and in some cases also of crystal aggregation. At low concentrations, 16µM, pyrophosphate inhibits calcium oxalate monohydrate crystal growth by 50%.[10] The urinary pyrophosphate levels (20-40µM) are theoretically high enough to inhibit calcium oxalate and calcium phosphate crystallisation. This was confirmed by Sharma et al who reported low 24-hour urinary excretion of pyrophosphate in stone formers as compared to non stone formers.[11]

**2.1.2. Organic inhibitors-** Osteopontin, Urinary prothrombin fragment 1 (UPTF1), Tamm-Horsfall protein, glycosaminoglycans, renal lithostathine, nephrocalcin etc. are some organic inhibitors shown in Table1, which inhibit the crystallization and are discussed in the following sub-sections.

**2.1.2.1. Osteopontin (Uropontin)**

This is a negatively-charged aspartic acid rich protein that inhibits growth of calcium oxalate crystals in a supersaturated solution. It is synthesized within the kidney and present in the human urine at levels in excess of 100 nM.[12] It is an abundant component of organic matrix of calcium oxalate stones. In vitro studies suggest osteopontin may inhibit nucleation, growth and aggregation of calcium oxalate crystals and also inhibits the crystal adhesion to renal epithelial cells. Some studies have reported decreased concentrations of OPN in urine of stone formers as compared to normal individuals.[13]

**2.1.2.2. Urinary prothrombin fragment 1 (UPTF1)**

This is a potent inhibitor of calcium oxalate stone formation in vitro.[14] The organic matrix of calcium oxalate crystals contains UPTF1, providing evidence that links the role of blood coagulation proteins with stone formation. UPTF1 is an important inhibitor of calcium oxalate crystal growth, aggregation and adherence of crystals to renal cells.[15]

**2.1.2.3. Tamm-Horsfall Protein (THP)**

Tamm-Horsfall protein (isolated by Tamm and Horsfall 50 years ago) is the most abundant glycoprotein in the urine of normal mammals. There is a lot of controversy about whether this protein is an inhibitor or promoter of kidney stone. Most believe it to be an effective inhibitor of calcium oxalate monohydrate crystals aggregation along with high pH, low ionic strength and low concentrations of divalent ions in a solution whereas low pH, high concentrations of calcium, sodium and hydrogen ion and low THP in a solution can act as promoters of aggregation.[16] THP has no inhibitory activity against crystal nucleation rather it promotes nucleation as shown in Table 2. It self aggregates into protein particles in high calcium and sodium, and low pH.[17]

**2.1.2.4. Glycosaminoglycans (GAGs)**

GAGs are enzymatic products of proteoglycans and have been identified as one

of the macromolecules present in the stone matrix.[18] They are believed to play an important role in calcium oxalate crystallisation. They have the ability to inhibit the growth and aggregation of calcium oxalate crystals by blocking the growth sites. They also prevent crystal adhesion to renal cells, which is an important step in renal stone formation.[19] However, no study shows qualitative or quantitative significant difference in total excretion of glycosaminoglycans between stone formers and non stone formers.

**2.1.2.5. Renal Lithostathine**

Renal Lithostathine, a protein of pancreatic secretion, is a urinary inhibitor of calcium carbonate crystal growth. Several studies showing the presence of calcium carbonate in renal stones suggested that crystals of calcium carbonate might be present in the early steps of stone formation.[20] Such crystals might therefore promote calcium oxalate crystallization from supersaturated urine by providing an appropriate substrate for heterogeneous nucleation.

**2.1.2.6. Nephrocalcin (NC)**

NC is composed of 110 amino acid residues of which 25% are glutamic and aspartic acid. It contains two cysteine and two or three γ-carboxyglutamic acid (Gla) residues which are suggested to play a significant part in its ability to inhibit calcium oxalate crystallization. One mole of Nephrocalcin binds 4 moles of $Ca^{2+}$ and its binding sites differ completely from those in other $Ca^{2+}$-binding proteins. Nephrocalcin is produced in human kidney by proximal tubule and the thick ascending limb of Henle's loop. Coe et al. isolated nephrocalcin that inhibits calcium oxalate crystal growth by absorption to crystal surfaces from urine, kidney tissue and calcium oxalate stones.[21] Nephrocalcin inhibits nucleation and aggregation of calcium oxalate stones and crystal adhesion to renal cells.[22]

**2.1.3. High urine volume**

One of the most important inhibitors of stone formation is high urine volume. A high volume of urine helps to reduce the relative supersaturation of the crystal forming components. Moreover, high volume indicates high urine flow rates, which tends to wash out any crystals formed already. One randomised clinical study going for five years showed stones recurred in only 12 out of 99 patients maintaining a urine volume of about 2.6L / day.[23]

**2.2 Promoters**

Urine contains substances that influence crystallization processes, and therefore regulate stone formation. Substances that increase crystallization are termed promoters. Low urine volume[24], low urine pH, calcium, sodium, oxalate, and urate are known to promote stone formation. On the cell surfaces of the kidney, cell debris, protein aggregates and other crystals may provide site for nucleation which might lower the supersaturation required to initiate crystallisation thereby promoting calcium oxalate crystallisation. There are strong geometric similarities between the crystals of uric acid dihydrate and calcium oxalate monohydrate which may promote overgrowth of one on the other.[25] Another factor that promotes the formation and growth of intrarenal crystals is ionic calcium. Hypercalciuria can decrease inhibitory activity and promotes crystallisation. Furthermore, cellular responses to newly formed crystals and factors that modulate these crystal-cell interactions could stimulate the formation of renal stone. The role of cell injury may be an even more important determinant in the promotion and progression of kidney stones.[26]

**3. CONCLUSION**

Renal stone formation is initiated by super saturation of urinary salts and crystal retention in the urinary tract. Stone formation is a multi-step process viz. nucleation, crystal growth; aggregation and retention are necessary steps in formation of stones. However, urine contains many substances that modify and inhibit the process of stone formation. Magnesium, pyrophosphate, citrate, nephrocalcin, osteopontin, glycosaminoglycans and Tamm-Horsfall protein are some substances that inhibit crystal aggregation. These substances play an important role in protecting normal subjects from stone formers. Abnormalities of these molecules might lead to stone formation i.e. a complex combination of inhibitors and promoters influence stone formation. Deficiency of inhibitors and an abundance of promoters in the urine facilitate stone formation, in addition to recognized dietary factors. The role of cell injury may be an even

more important determinant in the promotion and progression of kidney stones. Perhaps the presence of the stone itself initiates crystal growth leading to further epithelial disruption and progression of stone formation. All these considerations need further research in fields of assessment of role of inhibitors and promoters in renal stone formation.

**REFERENCES**


1. Biyani CS. The Role of Urinary Kidney Stone Inhibitors and Promoters in the Pathogenesis of Calcium Containing Renal Stones. J European Urology. 2007;5:126–136.
2. Smith CL. The medical aspects of Urolithiasis, an overview. J Urol (2). 1988;141;707-710.
3. 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
4. Robertson WG, Peacock M, Nordin BE. Inhibitors of the growth and aggregation of calcium oxalate crystals in vitro. Clin Chim Acta. 1973;43:31–37.
5. Francois B, Cahen R and Pascal B. Inhibitors of urinary stone formation in 40 recurrent stone formers. Br J Urol. 1986;58:479–483.
6. Kok DJ, Papapoulos SE and Bijvoet OL. Excessive crystal agglomeration with low citrate excretion in recurrent stone-formers. Lancet. 1986;1:1056–1058.
7. Desmars JF and Tawashi R. Dissolution and growth of calcium oxalate monohydrate. I. Effect of magnesium and pH. J Biochem.1973;313:256–267.
8. Liebman M and Costa G. Effects of calcium and magnesium on urinary oxalate excretion after oxalate loads. J Urol.2000;163:1565–1569.
9. Reungjui S, Prasongwatana V and Premgamone A. Magnesium status of patients with renal stones and its effect on urinary citrate excretion. BJU Int. 2002;90:635–639.
10. Schwille PO, Rumenapf G, Wolfel G and Kohler R. Urinary pyrophosphate in patients with recurrent calcium Urolithiasis and in healthy controls: a re-evaluation. J Urol. 1988; 140:239–245.
11. Sharma S, Vaidyanathan S, Thind SK and Nath R. Urinary excretion of inorganic pyrophosphate by normal subjects and patients with renal calculi in north-western India and the effect of diclofenac sodium upon urinary excretion of pyrophosphate in stone formers. Urol Int. 1992;48:404–408.
12. Worcester EM and Beshensky AM. Osteopontin inhibits nucleation of calcium oxalate crystals. Ann N Y Acad Sci. 1995;760:375–377.
13. Hoyer JR. Uropontin in urinary calcium stone formation. Miner Electrolyte Metab. 1994; 20:385-392.
14. Grover PK and Ryall RL. Inhibition of calcium oxalate crystal growth and aggregation by prothrombin and its fragments in vitro: relationship between protein structure and inhibitory activity. Eur J Biochem. 1999;263:50–56.
15. Webber D, Radcliffe CM and Royle L. Sialylation of urinary prothrombin fragment 1 is implicated as a contributory factor in the risk of calcium oxalate kidney stone formation. Febs J. 2006;273:3024–3037.
16. Huang HY and Zhu XH.Tamm-Horsfall protein is a critical renal defence factor protecting against calcium oxalate crystal formation. Kidney Int. 2004;66:1159–1166.
17. Fleisch H. Inhibitors and promoters of stone formation. Kidney International.1978;13: 361-371
18. Ryall RL. Glycosaminoglycans, proteins, and stone formation: adult themes and child's play. Pediatr Nephro. 1996;10:656–666.
19. Dussol B.Urinary Kidney Inhibitors. Where are we? Nephro Dial Transplant. 1996:11:1222-1224.
20. Tatemichi N, Kato M and Hayakawa S. Immunological characterization of pancreatic stone protein in human urine. J Clin Lab Anal.1994;8:76-80
21. Coe FL, Nakagawa Y, Asplin J and Parks JH. Role of nephrocalcin in inhibition of calcium oxalate crystallization and nephrolithiasis. Miner Electrolyte Metab.1994;20: 378-384

22. Mustafi D. and Nakagawa Y. Characterization of calcium-binding sites in the kidney stone inhibitor glycoprotein nephrocalcin with vanadyl ions: electron paramagnetic resonance and electron nuclear double resonance spectroscopy. Proc Natl Acad Sci USA. 1994;91:11323-11327
23. Hamm LL and Hering-Smith KS. Pathophysiology of hypocitraturic nephrolithiasis. Endocrinol Metab Clin North Am. 2002;31:885–893.
24. Coe FL, Parks JH and Asplin JR. The pathogenesis and treatment of kidney stones. N Engl J Med. 1992;327:1141–1152.
25. Lonsdale K. Epitaxy as a growth factor in urinary calculi. Nature. 1968;217: 56-58.
26. Miller NL, Evan A and Lingeman JE. Pathogenesis of renal calculi. Urologic Clinics of North America. 2007;34 (3):295-313.