(1981) Amino acids in urine and blood of calculous patients. J Urol 19:115
113. Walker RM, Linkswiler HM (1972) Calcium retention in the adult human male as affected by protein intake. J Nutr 102:1297
114. Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (1989) Urolithiasis. Plenum Press, New York
115. Weiss RE, Gorn A, Dux S, Nimni ME (1981) Influence of high protein diets on cartilage and bone formation in rats. J Nutr 111:804
116. Whang R, Welt LG (1963) Observations in experimental magnesium depletion. J Clin Invest 42:305
117. Williams G, Sallis JD (1982) Structural factors influencing the ability of compounds to inhibit hydroxyapatite formation. Calcif Tissue Int 34:169
118. Wilson DM, Smith LH, Erickson SB, Torres VE, Liedtke RR (1989) Renal oxalate handling in normal subjects and patients with idiopathic renal lithiasis: primary and secondary hyperoxaluria. In: Walker VR, Sutton RAL, Cameron ECB, Pak CYC, Robertson WG (eds) Urolithiasis. Plenum Press, New York, p 53
119. Wood RJ, Gerhardt A, Rosenberg JH (1987) Effects of glucose and glucose polymers on calcium absorption in healthy subjects. Am J Clin Nutr 46:699
120. Zarembski PM, Hodgkinson A (1962) The oxalic acid content of English diet. Brit J Nutr 16:627
121. Zechner O, Schreiber V (1985) Alcohol as an epidemiologic risk factor in urolithiasis. In: Schwille PO, Smith LH, Robertson WG, Vahlensieck W (eds) Urolithiasis and Related Clinical Research. Plenum Press, New York, p 315

Prof. Paul O. Schwille,
Universitäts-Krankenhaus,
Chirurgische Klinik,
W-8520 Erlangen, FRG

Urol Res (1992) 20:83-86

Tamm-Horsfall Glycoprotein - Inhibitor or Promoter of Calcium Oxalate Monohydrate Crystallization Processes?

B. Hess

Policlinic of Medicine, University Hospital, CH-3010 Berne, Switzerland

Summary. The processes of calcium oxalate monohydrate (COM) crystal nucleation, growth and aggregation (agglomeration) generally have been studied using a wide variety of assay systems/conditions. This paper reviews the apparently conflicting data on the effects of **Tamm-Horsfall glycoprotein (THP)** on COM crystallization processes in vitro, with the main emphasis on crystal aggregation. According to its well-known physico-chemical properties, THP has a dual role in modifying crystal aggregation: at high pH and low ionic strength (IS), THP is a powerful crystal aggregation inhibitor. Upon lowering pH and raising IS, THP viscosity increases, leading to reduced crystal aggregation inhibition. In the presence of additional calcium ions, some THPs even become strong promoters of crystal aggregation. This phenomenon seems to be more pronounced in THPs isolated from recurrent calcium stone formers whose proteins exhibit an abnormally high tendency of polymerization. Recent studies suggest an inherited molecular abnormality of THP among some severe recurrent calcium stone formers.

Key words: nephrolithiasis, calcium oxalate, inhibitors and promoters, Tamm-Horsfall glycoprotein

Introduction

When addressing crystallization within the urinary tract, two major aspects have to be considered (14): a **thermodynamic** one including high urinary supersaturation during which crystal nucleation occurs, and a **kinetic** one comprising rates of nucleation, growth and aggregation (agglomeration) of crystals. When reviewing studies on urinary compounds that modify calcium oxalate crystallization kinetics, a somewhat confusing picture emerges. In many studies on **"crystallization"**, it is often not clear whether authors refer to nucleation, growth or aggregation of crystals, and a wide variety of **assay systems/conditions** is being applied for measuring these processes. Furthermore, the term **"inhibitor"** has been used for compounds that act as chelators of calcium or oxalate ions as well as for molecules binding to the surface of preformed calcium oxalate crystals (17). Whereas **chelators** reduce free ion activity and, therefore, supersaturation, "real" **inhibitors** do not influence supersaturation, since they bind to crystal surfaces and block growing sites ("crystal poisoning") at very low (usually micromolar) concentrations (1). Additionally, some molecules may act as **promoters**, probably by providing preformed surfaces

for heterogeneous nucleation, epitaxial growth and aggregation of crystals (1). Since urinary compounds can have such varying effects on different crystallization processes, the term **"modifier"** should be used (23), and it should always be stated whether a specific molecule inhibits or promotes nucleation, growth or aggregation of crystals.

Tamm-Horsfall glycoprotein

The pathophysiology of Tamm-Horsfall glycoprotein (THP) - also called **uromucoid** - has been reviewed extensively (12, 15, 18). THP is a glycoprotein produced by the kidneys; its carbohydrate content amounts to 30%. In its monomeric form, THP has a molecular weight (MW) of about 80 kD (15). The exact function of THP remains enigmatic (12, 15, 18), but recent studies suggest that THP might be a specific ligand for cytokines in the kidney (15).

Because THP is present in various amounts in renal stones (7), it has been proposed to play a role in **renal stone formation**. In several studies no difference was found between normals and calcium oxalate stone formers in terms of daily urinary excretion of THP, averaging 40-50 mg (2, 7, 21, 26); in patients with distal renal tubular acidosis, however, THP excretion seems to be significantly lower than in controls (25).

The results of available studies on THP effects on calcium oxalate crystal nucleation, growth and aggregation seem very contradicting. Kitamura and Pak reported 19% inhibition of calcium oxalate **crystal nucleation** by 50 mg/l of normal human THP at pH 6.5, ionic strength (IS) 0.15 and 1 mM $CaCl_2$ (13). Lowering pH to 6.0, however, produced 25% promotion of nucleation (29). In the presence of very high calcium (6.76 mM) and oxalate (1.03 mM) concentrations at pH 5.7 in a synthetic urine, THP at only 1 mg/l promoted nucleation by 183% (4). The addition of 35 mg human THP to 1 liter of extremely concentrated urine (evaporated to 1250 mOsmole/kg) at pH 5.3 promoted the nucleation of amorphous calcium oxalate crystals by 250% (19)!

Most authors agree that THP is a weak inhibitor of calcium oxalate **crystal growth** in vitro (13, 28, 29). At pH 6.5 and IS 0.15, 50 mg/l of THP inhibited by 38% in the pesence of calcium and oxalate at 0.44 mM each (13). At pH 6.0, IS 0.15 and calcium and oxalate concentrations of 1 mM and 0.2 mM, respectively, 50 mg/l of THP inhibited by 3% (29), whereas 8 mg/l did not affect crystal growth (28).

Measuring calcium oxalate **crystal aggregation (agglomeration)** in vitro, Scurr and Robertson found 22% inhibition by 48 mg/l of isolated THP at pH 6.0, IS 0.15, 2 mM calcium and 0.4 mM oxalate (22). Upon addition of oxalate to metastably supersturated ultrafiltered urine from healthy humans in the presence of isolated THP (50 mg/l), predominantly small COM crystals, but not aggregated calcium oxalate dihydrate "envelopes" are formed (20). Using the newly developed spectrophotometric method at pH 7.2, IS 0.1 and only equilibrium concentrations of calcium and oxalate (saturated conditions), we found 90% aggregation inhibition by normal human THP at 40 mg/l (8). In a subsequent study, however, lowering pH to 5.7 and increasing IS to 0.21 at otherwise unchanged conditions revealed 35% promotion of aggregation by 40 mg/l of THP isolated from calcium renal stones (9). More recently, studying the effects of 5 mM calcium at pH 5.7 and 200 mM NaCl (11), we were able o demonstrate that 4 out of 5 THPs from severely recurrent stone formers promoted COM crystal aggregation (inhibition between -68% and +7%), whereas 6 THPs from healthy controls had either no effect or inhibited aggregation (inhibition between -3% and +50%).

These apparently paradoxical influences on the various crystallization processes may be explained by the well-known **physicochemical properties of THP**: increasing the concentrations of the protein itself (16), divalent cations like calcium and magnesium (3, 24), sodium (16, 24) and hydrogen ions (16) all increase polymerization of THP molecules, leading to reversible gel formation (increased viscosity) (6). Scanning electron microscopy studies revealed that THP molecules formed elongated fibers about 20 nm thick and up to 1000 nm long in solutions containing NaCl (100 mmol/l) or $CaCl_2$ (1 mmol/l); when NaCl and $CaCl_2$ were simultaneously present, however, thick bundles of fibers could be seen, like those found in hyaline casts from human urine (27). It is obvious from all available studies that reduced THP inhibition or even promotion of calcium oxalate crystallization processes always occurred at lower pH and higher IS.

Studying the effects of increasing IS from 0.01 to 0.21 at pH 5.7 on individually purified THPs (40 mg/l) from normal men and male patients with severe recurrent idiopathic calcium oxalate stone disease (>20 stones), we found that COM crystal aggregation inhibition was reduced more markedly in stone former THPs (10). This seems to be the consequence of a **molecular abnormality** of stone former THP, which exhibits an increased tendency to polymerize at lower pH and higher IS, as evidenced from viscosity measurements and molecular weight determinations (10). Studies on THPs of the family members of one severe stone former indicate that this apparent abnormality, the molecular basis of which remains unknown, might be **inherited** (10).

Most authors agree that the **mechanism** by which urinary macromolecules inhibit crystal growth and aggregation in vitro, is their binding to the crystals, which induces a more negative surface charge (Zeta potential) on the crystal surface (5, 22). In comparison with glycosaminoglycans and RNA, however, THP produces a less negative Zeta potential on COM crystals (22). Furthermore, two studies have demonstrated that upon raising THP concentrations within physiologic urinary limits, e.g. above 10^{-7} M, Zeta potential does not become more negative, e.g. the potential curve flattens off at about -20 mV (8, 22). Our own studies did not reveal Zeta potential differences between THPs from healthy men and severely recurrent stone formers, nor was COM crystal aggregation inhibition by physiologic THP concentrations correlated to Zeta potential values (10). We found, however, a negative linear correlation between crystal aggregation inhibition and **intrinsic viscosity** of THP at pH 5.7 and 200 mM NaCl, e.g. THPs with lower viscosities allowed for more crystal aggregation

inhibition (9, 10).

Altogether, there is strong evidence for a **dual role of THP** in COM crystal aggregation. At higher pH and lower IS, THP is a powerful aggregation **inhibitor**. Upon lowering pH and raising IS within physiologic urinary limits, marked polymerization of THP molecules occurs. This most probably increases attractive viscous binding forces on COM crystal surfaces. Since the repulsive Zeta potential is not increased any further, the overall forces between crystals become more attractive (5), allowing for more crystal aggregation (reduced inhibition). If in addition physiological calcium concentrations are present at low pH and high IS, certain THPs even become **promoters** of COM crystal aggregation.

Acknowledgement. Dr. Hess is a grant recipient of the Swiss National Science Foundation (No. 32-26428-89).

References

1. Baumann JM (1988) How reliable are the measurements of crystallization conditions in urine? (Editorial). Urol Res 16:133-135
2. Bichler KH, Kirchner CH, Ideler V (1976) Uromucoid excretion of normal individuals and stone formers. Brit J Urol 47:733-738
3. Cleave AJ, Kent PW, Peacocke AR (1972) The binding of hydrogen and calcium ions by Tamm-Horsfall glycoprotein. Biochim Biophys Acta 285:208-223
4. Drach GW, Thorson S, Randolph A (1980) Effects of urinary organic macromolecules on crystallization of calcium oxalate: enhancement of nucleation. J Urol 123:519-523
5. Finlayson B (1978) Physicohemical aspects of urolithiasis. Kidney Int 13:344-360
6. Fletcher AP (1972) The Tamm and Horsfall glycoprotein. In: Gottschalk A (ed.) Glycoproteins: Their Composition, Structure and Function (2nd ed.). Elsevier, New York, part B, chapt. 7:892-908
7. Grant AMS, Baker LRI, Neuberger A (1973) Urinary Tamm- Horsfall glycoprotein in certain kidney diseases and its content in renal and bladder calculi. Clin Sci 44:377-384
8. Hess B, Nakagawa Y, Coe FL (1989) Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. Am J Physiol 257 (Renal Fluid Electrolyte Physiol 26):F99-F106
9. Hess B, Nakagawa Y, Kim B, Coe FL (1989) Functional heterogeneity of Tamm-Horsfall glycoprotein isolated from urines of normal subjects and idiopthic calcium oxalate stone formers and from human calcium oxalate kidney stones. In: Walker VR et al. (eds) Urolithiasis, Plenum Press, New York:173-176
10. Hess B, Nakagawa Y, Parks JH, Coe FL (1991) Molecular abnormality of Tamm-Horsfall glycoprotein in calcium oxalate nephrolithiasis. Am J Physiol 260 (Renal Fluid Electrolyte Physiol 29): F569-F578
11. Hess B (1991) The role of Tamm-Horsfall glycoprotein and nephrocalcin in calcium oxalate crystallization processes. Scanning Microsc (in press)
12. Hoyer JR, Seiler MW (1979). Pathophysiology of Tamm-Horsfall protein. Kidney Int. 16:279-289
13. Kitamura T, Pak CYC (1982) Tamm and Horsfall glycoprotein does not promote spontaneous precipitation and crystal growth of calcium oxalate in vitro. J Urol 127:1024-1026
14. Kok DJ, Papapoulos SJ, Bijvoet OLM (1990) Crystal agglomeration is a major element in calcium oxalate urinary stone formation. Kidney Int 37:51-56
15. Kumar S, Muchmore A (1990) Tamm-Horsfall protein - uromodulin (1950-1990). Kidney Int 37:1395-1401
16. McQueen EG, Engel GB (1966). Factors determining the aggregation of urinary mucoprotein. J Clin Path 19:392-396
17. Robertson WG, Scurr DS, Bridge CM (1981) Factors influencing the crystallization of calcium oxalate in urine - critique. J Cryst Growth 53:182-194
18. Ronco P, Brunisholz M, Geniteau-Legendre M, Chatelet F, Verroust P, Richet G (1987). Physiopathologic aspects of Tamm- Horsfall protein: a phylogenetically conserved marker of the thick ascending limb of Henle's loop. Adv Nephrol 16:231-250
19. Rose GA, Sulaiman S (1982) Tamm-Horsfall mucoproteins promote calcium oxalate crystal formation in urine: quantitative studies. J Urol 127:177-179
20. Ryall RL, Harnett RM, Hibberd CM, Marshall VR (1989) The effect of macromolecules on the crystallization of calcium oxalate in human urine. In: Walker VR et al (eds) Urolithiasis. Plenum Press, New York:133-135
21. Samuell CT (1979) Uromucoid excretion in normal subjects, calicum stone formers and in patients with chronic renal failure. J Urol Res 7:5-12.
22. Scurr DS, Robertson WG (1986) Modifiers of calcium oxalate crystallization found in urine. II. Studies on their mode of action in artificial urine. Urol 136:128-131
23. Smith LH (1987) Pathogenesis of renal stones. Mineral Electrolyte Metab 13:214-219
24. Stevenson FK, Cleave AJ, Kent PW (1971) The effect of ions on the viscometric and ultrastructural behaviour of Tamm-Horsfall glycoprotein. Biochim Biophys Acta 236:59-66
25. Strohmaier WL, Bichler KH, Korn S, Wilbert DM (1990) Risk factors for stone formation in renal tubular acidosis. In: Urolithiasis, Proceedings of the 1st European Symposium on Urolithiasis (eds. Vahlensieck W et al.):38-40, Excerpta Medica, Amsterdam
26. Thornley C, Dawnay A, Cattell WR (1985) Human Tamm-Horsfall glycoprotein: urinary and plasma levels in normal subjects and patients with renal disease determined by a fully validated radioimmunoassay. Clin Sci 68:529-535
27. Wiggins RC (1987) Uromucoid (Tamm-Horsfall glycoprotein) forms different polymeric arrangements on a filter surface under calcium oxalate - Tamm-Horsfall glycoprotein & Nephrocalcin different physicochemical conditions. Clin Chim Acta 162:329-340
28. Worcester EM, Nakagawa Y, Wabner CL, Kumar S, Coe FL (1988) Crystal adsorption and growth slowing by nephrocalcin, albumin, and Tamm-Horsfall protein. Am J Physiol 255 (Renal Fluid

Electrolyte Physiol 24):F1197-F1205
29. Yoshioka T, Koide T, Utsunomiya M, Itatani H, Oka T, Sonoda T (1989) Possible role of Tamm-Horsfall glycoprotein in calcium oxalate crystallization. Brit J Urol 64:463-467

Bernhard Hess, M.D.
Medizinische Universitätspoliklinik
Inselspital Bern
CH-3010 Bern, Switzerland

Urol Res (1992) 20:86-88

Urolithiasis, Inhibitors and Promoters

F. Grases and A. Conte

Laboratory of Urochemistry and Crystallization, Dpt. Chemistry, University Illes Balears and Urolithiasis section, Hospital Son Dureta, Palma de Mallorca, Spain

Summary. The aim of this work is to evaluate the role and importance of inhibitors and promoters in urolithiasis. Carrying in mind theoretical considerations, we conclude that in urolithogenic processes, inhibitors and promoters could only play a decisive role in the "idiopathic" oxalocalcic urolithiasis. We classify the "idiopathic" oxalocalcic stone-formers into three main groups, considering inhibitory and promoting factors. It is shown that such classification is in good agreement with the clinical results observed in a group of 88 "idiopathic" oxalocalcic stone-formers.

Key words: Urolithiasis, promotion, inhibition

Introduction

Many factors have been advanced to explain formation of stones in the urinary tract. In recent years, an important role seems to be assigned in general to the so-called inhibitors and promoters of the crystallization (1, 2). Nevertheless, it must be considered that in the formation of crystalline masses, both factors inhibitors (nucleation or crystal growth) and promoters (basically, heterogeneous nucleants), only can be decisive when being in non-excessive supersaturation. It must be considered that at high supersaturation the nucleation is homogeneous and crystal growth could take place mainly controlled by surface nucleation or by diffusion, and consequently inhibitors would cause little effect or even cause no effect. Thus, in urolithogenic processes, inhibitors and promoters could only play a decisive role in the "idiopathic" oxalocalcic urolithiasis (nor hypercalciuria, nor hyperoxaluria). It must be taken into account that, generally, in uric, phosphatic and cistinic urolithiasis important degrees of supersaturation are found. The object of this report is to demonstrate the role and importance of inhibitors and promoters on "idiopathic" oxalocalcic urolithiasis.

Material and Methods

Our study included 88 patients with calcium oxalate urolithiasis, without hypercalciuria (excretion of calcium < 250 mg/24 h) and hyperoxaluria (excretion of oxalate < 40 mg/24 h). In all cases the group of subjects was selected to comprise an equal number of women and men, and ages between twenty and sixty-five years. All subjects were on free diets at the time of urine collection and none of the stone-formers were undergoing pharmacologic treatment of any kind. All of them had been subjected to metabolic evaluation, including calcium, oxalate, uric acid and citrate. Urinary calcium was determined by atomic absorption spectroscopy; uric acid, citrate and oxalate by the Boehringer Mannheim kits No. 704156, 139076 and 755699, respectively. Composition of the calculus was determined for each patient by infrared spectroscopy.

Results and Discussion

In accordance with our experience, considering "in vivo" and "in vitro" results, the most important inhibitors and promoters of "idiopathic" calcium oxalate urolithiasis appear in Table 1. Taking into account the above mentioned results, we propose the classification of the "idiopathic" oxalocalcic stone-formers into three main groups.

Thus, in a first group we include individuals with tendency to urinary pH < 5.5 (favoured by Protein rich, lipid rich and glucid rich diets). In this group promotion would be clearly favoured by heterogeneous nucleation on uric acid and the inhibition of calcium oxalate crystallization disfavoured due to low citrate excretions (as a consequence of acidic blood pH). It is also possible that in some cases the presence of low urinary glycosaminoglycans concentrations favour the heterogeneous nucleation of calcium oxalate on uric acid.

In a second group, we include the individuals with tendency to urinary pH values between 5.5 - 6.5