BEHAVIOUR OF TAMM-HORSFALL PROTEIN (THP) IN LITHOGENIC PROCESS : I CLINICAL STUDIES

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ABSTRACT

The aim of the study was to measure urinary excretion of Tamm–Horsfall protein (THP), an important inhibitor or promoter of crystallization. The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes-nucleation, crystal growth, crystal aggregation and crystal retention.

Various aetiological factors have been attributed to stone formation-heriditary,dietary,geographical,infective etc. Approximately 85% of the stones in human are calcium stones comprising oxalate and phosphate ,either alone or combined.Though supersaturation of stone forming salts in urine is essential, abundance of these salts by itself will not always result in stone formation.

To access the influence of THP, isolated from NS and SF, an in vitro experiment was planned. Urine samples of the normal subjects (NS) and stone formers (SF) were collected and pooled together separately . For this purpose THP isolated from NS and SF in different concentration then no changes was observed in magnitude of calcium, oxalate and phosphate crystallization. In both the groups calcium salt crystallization increases with increase in concentration of THP. When THP isolated from SF showed higher crystallization of these three promoters.

In conclusion ,the data suggested that THP should behave as a promoter and greater risk of recurrence of stone disease.

KEY WORDS

Tamm-Horsfall protein, Uromodulin, Calcium oxalate, Stone Formers. promoters, inhibitors.

INTRODUCTION

Tamm-Horsfall Protein (THP) also referred as uromodulin , 78-80 kd glycoprotein has been detected in kidney,liver,heart,muscles,lungs,brain ,thymus and spleen. It is excreted in the range of 20-200 mg/day in

human urine and is the major constituent of the cast (Dussol and Berland 1998 and Kumar and Muchmore,1990).The native THP is a weak inhibitor of calcium oxalate nucleation and growth (Dussol and Berland 1998;Chan et al. 2012 and Walaa et al.2014). Its role in urine is mainly reported to be promoter (Grover et al. 1989;Rose 1987 and Rose and Sulaiman, 1985), though some have reported to be inhibitor (Sophason et al. 1980) and few have observed no influence. Our studies have consistently indicated to be promoter.The present study further confirm our earlier findings.

METHODS

The 24 hr. urine samples of the normal subjects (NS) and stone formers (SF) were collected and pooled together seperately.THP from these samples was isolated by the method described by Tamm and Horsfall (1950) and determined by Natelson (1971). The crystallization (mineralization) on 3mm glass fibres was determined by the method of Sur et al. (1987). We used 3mm glass fibres instead of 0.3mm fibres. To access the influence of THP isolated from NS and SF, an in vitro experiment was planned. Pooled native urine samples from NS and SF were collected. THP in the concentration of 15mg/l and 30mg/l was added in these native urine samples and crystallization potential was determined.

RESULTS

Table -I show data on changes of calcium, oxalate and phosphate crystallization on NS and SF with and without THP addition isolated from NS . In both the group THP showed progressive increase in calcium salts crystallization with increase in concentration . Notably the magnitude of crystallization was almost same in both the group. On the contrary ,the THP isolated from SF showed much higher crystallization of these three promoters and the magnitude of crystallization was much higher in SF (Table- II).The urine sample collected from bladder by catheter showed similar trend (given in parentheses).

Table- I Effect of in vitro addition of Tamm-Horsfall Protein isolated from normal subjects to urine of normal subjects and stone formers on calcium ,oxalate and phosphate crystallization

Parameter	Normal Subjects (n=30)					Stone Formers (n=30)				
	No addition	15mg/l	Increase in deposition	30mg/l	Increase in deposition	No addition	15mg/l	Increase in deposition	30mg/l	Increase in deposition
Calcium(µgm)	124± 14 (130± 16)	132 ±22 (134 ± 18)	8± 7 (4± 3)	141± 23 (138± 15)	17± 9 (8± 7)	371± 102 (315 ±106)	378 ±107 (325 ± 72)	7± 4 (10± 6)	400 ±116 (356± 110)	28± 13 (41± 15)
Oxalate(µgm)	102 ±21 (106± 15)	111 ±22 (110 ± 18)	9 ±1 (4± 2)	123± 18 (118± 14)	21± 2 (12± 2)	168 ±34 (173± 38)	174± 35 (181 ± 28)	8± 1 (8± 2)	186 ±35 (180± 30)	18 ±1 (7± 2)

Phosphate(µgm)	50± 11 (46 ±12)	59± 11 (56± 15)	9± 1 (10± 2)	68± 7 (66 ±9)	17± 4 (20 ±4)	96± 21 (89± 20)	109± 19 (106 ± 14)	12± 2 (17± 3)	117 ±25 (110± 18)	21± 3 (21± 4)

Effect on urine sample collected from bladder by catheter (n=6) is given in parentheses

Table- II Effect of in vitro addition of Tamm- Horsfall Protein isolated from stone formers to urine of normal subjects and stone formers on calcium ,oxalate and phosphate crystallization

Parameter	Normal Subjects (n=30)					Stone Formers (n=30)				
	No addition	15mg/l	Increase in deposition	30mg/l	Increase in deposition	No addition	15mg/l	Increase in deposition	30mg/l	Increase in deposition
Calcium(µgm)	124± 14 (112± 16)	145 ±20 153± 24	21± 6 (41± 8)	161± 22 (192 ± 26)	37± 8 (80± 12)	371± 120 (360 ±115)	408 ±120 (401 ± 98)	87± 17 (41± 15)	443±120 (467± 125)	71± 17 (107± 21)
Oxalate(µgm)	102 ±21 (104± 18)	129 ±25 125± 21	27 ±4 (21± 5)	144± 17 (158 ± 18)	42± 3 (54± 10)	168 ±34 (170± 41)	195± 40 (218 ± 32)	27± 6 (48± 10)	209 ±29 (214± 34)	40 ±4 (44± 7)
Phosphate(µgm)	50± 11 (35 ±12)	71± 17 68± 10	39± 6 (33± 9)	78± 10 (89 ±16)	47± 1 (54 ±6)	96± 21 (98± 15)	131± 22 (106 ± 24)	35± 1 8± 3	145±27 (148± 20)	48± 6 (50± 11)

Effect on urine sample collected from bladder by catheter (n=6) is given in parentheses

DISCUSSION

Kumar and Muchmore (1990) comprehensively reviewed the role of THP in different diseases including urolithiasis and concluded that about 30% carbohydrate present in this glycoprotein is equal partner with protein on their influence on biological system , primary on kidney. Their own studies indicated that THP has no effect on calcium oxalate crystal growth(Monika Gupta et al.2011 and M. Carvalho et al.2018) and preferred to suggest that it could be an innocent bystander in lithogenic process and might be getting emeshed in renal stones by coincidence. Contrary to this belief many authers agree that THP is a mild inhibitor in its monomeric form which is present in concentration of 98% whereas is a strong promoter in aggregated form present at higher concentration with a high ionic strength and lower PH .These observations imply that in native urine THP should behave as a promoter. The observation of Rose and Sulaiman (1985) and Scurr and Robertson (1986) lend support to this contention. Our observations are also in their agreement .These observations are further strengthened by this study.

We agree that increase in urinary THP levels in SF is not an universal phenomenon but results give an strong inkling that SF with an increased THP excretion are at a greater risk of recurrence of the disease due to its promoting effect. At present cognitive measures are not available to manipulate THP excretion except in cases suffering from severe infection. Therefore, studies in this direction are much needed.

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BEHAVIOUR OF TAMM-HORSFALL PROTEIN (THP) IN LITHOGENIC PROCESS : I I ANIMAL STUDIES

INTRUCTION

Indeed hyperoxaluria (Robertson,1999 and Singh and Srivastava 1992) and hypercalciuria (Coe and Parks 1999) are ultimate determinant of calcium oxalate lithogenesis but there are a number of organic and inorganic molecules which modulate this process.Tamm-Horsfall Protein (THP) is one of them.Various studies amply indicate that THP can behave as urinary stone promoter or inhibitor depending on PH and ionic concentration.At high PH ,low ionic strength and low divalent cation concentration.THP exist in monomeric form and act as inhibitor of calcium oxalate aggregation.In converse situation THP , depending upon the magnitude of conditions, under goes progressive reversible polymerization forming gel of various consistencies.This gel has a tendency to entrap calcium oxalate/phosphate crystals and this tendency increases with increasing concentration of aggregated THP. While we largely agree with these observations but what we want to emphasize is: that in urinary environment as it exist in the local population a part of THP is always present in aggregated form ; always overshadows inhibitory activity ; and that the promoting effect of THP is more in stone formers. We have already demonstrated this in another paper in human urine presented in this symposium and elsewhere (Singh et al.1987) and now we further support this claim by animal studies.Gokhale et al. (1998)have demonstrated that urinary THP from many animal is comparable to human urinary THP.

METHODS

Eight guinea pigs were made hyperoxaluric by feeding ethylene glycol (EG) in drinking water (0.25%) and injecting gentamycin (GM) subcutaneously (100mg/kg BW).The treatment was given for 15 days and then animal continued on standard diet and water ad libytum. Urine samples were collected before treatment and then on day 5,10,15 and 25. Calcium (Kit method),oxalate (Hodgkinson and Williams,1972),THP (Tamm-Horsfall,1950) and TBAR (Buege and Aust,1978) levels in urine were determined.On day 30 the urine samples of all eight animals was collected for 24 hours and pooled together.This pooled urine sample was split into two. In one sample THP isolated from hyperoxaluric animals (on day 12) and in the other isolated from normal animal maintained in animal house was added. The calcium oxalate and phosphate crystallization was determined by method of Sur et al. (1987). We used 3mm glass fibres instead of 0.3mm glass fibres and maintained uniform temperature (37 degree centigrade) during the experiment.

RESULTS

The effect of EG+GM on urinary oxalate THP and TBAR is given Table- I. The data on plasma TBAR level are also incorporated in same table. The oxalate level increased from 1.89± 0.17 mg/24hrs. to 4.11± 0.11 mg/24hrs. THP showed significant but only mild rise in its concentration. As expected both urinary and plasma TBAR levels were raised considerably. On day 25 the urine chemistry showed tendency to revert to normal profile, though plasma TBAR levels were appreciably high but showed decline. The treatment regimen distinctly increased crystallization of calcium oxalate and phosphate which became almost on day 25 indicating that the stone risk created was reversible (Table -II).Importantly in the urine

sample in which THP from hyperoxaluric animal was added, the crystallization of calcium was almost double compared to the one in which THP isolated from normal animals was added. Further this enhanced crystallization of calcium appears to be as calcium oxalate rather than phosphate.

Table-I 24 hour urine excretion and plasma TBAR levels.

Urine	Before Trt.	Trt.(EG+GM)			After Trt.
		Day 5	Day 10	Day 15	Day 25
Oxalate(mg)	1.89± 0.17	2.41± 0.22*	3.18 ±0.30*	4.11± 0.11*	1.90± 0.21
THP(mg)	8.05 ±0.17	8.39± 0.25*	9.55± 0.39*	10.10 ±0.34*	8.26± 0.32
TBAR(μ mol)	0.145± 0.019	0.182 ±0.013*	0.294 ±0.010*	0.412± 0.011*	0.173± 0.10
Plasma TBAR(nmol/ml)	1.26± 0.14			3.51± 0.12*	2.10± 0.14*

EG=Ethylene glycol, GM= Gentamycin *p<0.05

Table-II Urinary crystallization of calcium ,oxalate and phosphate on different regimen.

Parameters	Before Trt.	Trt.(EG+GM)			After Trt.	In vitro addition of THP(10mg/L) in urine collected on Day 30	
		Day 5	Day 10	Day 15	Day 25	THP from hyperox.animals	THP from normal animals
Calcium(μ g)	91± 6	165± 21*	246± 25*	333± 28*	98± 9	158 ±19	88± 11
Oxalate(μ g)	75± 5	119± 27*	168± 26*	249± 24*	77 ±5	109± 19	72± 6
Phosphate(μ g)	35± 4	45± 5*	61± 8*	69 ±4*	37± 4	39± 7	36± 6

EG=Ethylene glycol, GM= Gentamycin *p<0.05

DISCUSSION

THP is primarily distributed in kidney though renal environment is not necessary for the transcription of THP gene. Within the kidney THP is mainly confined to epithelial cells of the thick ascending limbs of loop of Henle and proximal part of the distal convoluted tubule. While several physiological functions have been attributed to THP in kidney, the two important functions in reference to urolithiasis are : a) urothelial defence against infection and, b) blocking the access of the crystal to the cell surface. In both of these events THP behaves as inhibitor and is in monomeric form. However under changing environment of urinary milieu and PH ,while passing through urinary tract about 2% monomeric THP is converted to polymeric form. Though its percentage is very minor yet the sum of total behavior of THP becomes promoter . The decrease in urine PH further aggravates its promoter activity. Our results in the series are testimony to this statement.The THP level progressively but slightly increased with ethylene glycol and

gentamycin but at the same time calcium and oxalate crystallization tripled. Admittedly this crystallization represents sum of supersaturation created by promoters but THP is one of the contributors of this promoter activity is strengthened by next experiment . THP isolated from hyperoxaluric urines showed implicity higher crystallization than isolated from normal urine.

These observations also explain why variable data on urinary excretion of THP in normal subjects and stone formers are reported in literature. Perhaps this variation is related to some other pathophysiology rather to lithogenic process and has mistakenly been associated with urinary THP levels. Indeed, further focus is now necessary to elucidate the nature , chemical composition and configuration of THP in native human urine and in what way it differs from that present within the kidney cells of different region or elsewhere performing other functions.

Large number of studies including ours have shown that EG induces hyperoxaluria (Singh et al. 1998). Some of these studies also indicate that the enlarged oxalate pool either by EG or other sources induces oxidant stress and that the later causes the mortality of renal tubular epithelial cells providing nidus for stone birth and growth . To examine the practical aspect of it, GM was administered along with EG to induce additional oxidant stress . The animal sacrificed after the experiment did not reveal presence of concretion or crystals in urinary tract ,suggesting that short term moderate to severe oxidant stress does not lead to stone formation. Therefore ,long term elaborate and conclusive animal and human studies are necessary to elicit the influence of oxidant stress on etiopathogenesis of urinary calculi before we interpret and apply the results of in vitro studies reported by Scheid et al. (1996) and others in human system.

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