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# ROLE OF TAMM-HORSFALL PROTEIN (THP) IN LITHOGENIC PROCESS : ANIMAL STUDIES

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**ABSTRACT**

One of the defenses against nephrolithiasis is provided by macromolecules that modulate the nucleation, growth, aggregation and retention of crystals in the kidneys. 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 rasing ionic strength THP viscosity increases, leading to reduced crystal aggregation inhibition. For this purpose eight guinea pigs were made hyperoxaluric. The treatment was given for fifteen days; then urine samples were collected before treatment ; then on 5,10,15and 25 day( after treatment) and in vitro addition of THP on 30th day which was isolated from hyperoxaluric and normal animals. The effect of EG+GM on urinary oxalate, THP and TBAR levels increases but after treatment the urine chemistry revert to normal profile, though plasma TBAR levels were appreciably high. The crystallization of calcium was almost double when THP was isolated from hyperoxaluric animals rather than normals. Our study suggested that THP act as a promoter.

## INTRODUCTION

Indeed hyperoxaluria (Robertson, 1999 and Singh and Srivastava 1992) and hypercalciuria (Coe and Parks 1999) are ultimate determinant of calcium oxalate lithogenesis(David T.Tzou T.et al.2016) but there are a number of organic and inorganic molecules which modulate this process. Tamm - Horsfall Protein (THP) is one of them. THP, a glycosylphosphatidylinositol glycoprotein, the most abundant urinary protein(Tsai-Hung Wu et al.2018)produced by renal tubular cells in thick ascending limb of loop of Henle. A 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 an inhibitor of calcium oxalate aggregation (Dussol and Berland 1998;Chan et al. 2012 and Walaa et al.2014). 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 Treatment | Treatment (EG+GM) | | | After Treatment |
|---|---|---|---|---|---|
| | | 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 Treatment | Treatment (EG+GM) | | | After Treatment | 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. THP has no effect on calcium oxalate crystal growth(Monika Gupta et al.2011 and M. Carvalho et al.2018) 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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