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OXALATE BINDING ACTIVITIES OF CALCIUM OXALATE MONOHYDRATE BINDING PROTEINS IN UROLITHIASIS UPON SUPPLMENTATION OF ANTIOXIDANT VITAMIN E

Medical Science

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ABSTRACT

The effect of vitamin E in thiol status and oxalate binding activity was studied in urolithic patients. Oxalate binding activity and calcium oxalate crystal deposition were markedly pronounced in stone patients. The oxalate binding activities of the COM binding proteins were assayed in all three fractions(F I and F II – pH 7.4; F III – pH 4.5). All the three fractions had oxalate binding activity when compared to control. Fraction I isolated from kidney stone formers exhibited 78.35% increase in with oxalate binding activity [p<0.001] .Vitamin E supplementation to renal stone formers for a follow up peroid of 9 months normalised the thiol status and oxalate binding activities of the COM binding proteins.

KEYWORDS

Urolithiasis,Calcium Oxalate Monohydrate,Thiol,Vitamin E

INTRODUCTION:

Urinary stone disease has afflicted mankind since antiquity and it persists with serious medical consequences, throughout a patient's lifetime. Most calculi in the urinary system arise from components of urine of which calcium oxalate (CaOx) represents up to 80 % of the analyzed stones (1). Renal calcium binding proteins have been identified in several biological systems (2). Urolithiasis is an art sculpted by the protein architects of the urine, with calcium and oxalate as building blocks and during the process, the architects themselves act as glue and bind the blocks more tightly. In the present study, the therapeutic efficacy of vitamin E therapy was assessed using the thiol status and oxalate binding activities of Calcium Oxalate Crystal binding proteins.

PATIENTS AND METHODS

This study was approved by the institutional ethical committee.. The patients were also given a clear picture of the benefical effct of vitamin E (400 mg)

Normal subjects and Kidney stone patients (KSP) without any complications were included in the present study. Patients admitted in the Urology ward of Stanley Medical College & hospital and confirmed for the presence of stones by x- rays, KUB scan and intravenous ureterogram (IVU) and had undergone surgery for the removal stones were considered as stone formers.

EXPERIMENTAL DESIGN

Group I - Normal healthy individuals (control)
Group II a - Stone patients
Group Iib - Stone Patients supplemented with vitamin E for 3 months
Group Iic - Stone Patients supplemented with vitamin E for 6 months
Group IId - Stone Patients supplemented with vitamin E for 9 months

METHODS

24 hrs urine samples were collected from stone formers before supplementation of vitamin E and at three months interval for the duration of nine months after supplementation.Vitamin E supplementation was given along with their regular treatment regime.
COM binding proteins were isolated by the method follwed by Kalaiselvi etal.(3).COM crystals were freshly prepared before use by mixing 1.5M $CaCl_2$ and 0.3M potassium oxalate in the ratio of 1:5 (adjusted to pH 6.5 using Tris – HCl buffer) with constant shaking at room temperature.After 30 minutes of stabilization of the system by agitation. 0.3 potassium chloride was added and the solution was made upto twice the initial volume.protein (3 mg protein / mg crystal) was added and allowed to interact with the proteins with constant shaking of the solution for 1 hour. The solution was centrifuged at 4000 g for 10 minutes and precipitated calcium oxalate was washed with water thrice for removal of the extraneously bound protein. 25mM Ethylene diamine tetra acetic acid(EDTA) was used for extraction of the bound protein. EDTA extract was separated by centrifugation at 4C at 10000 g for 10 minutes and dialyzed against water at 4C overnight with two changes of water.Then isolated COM binding proteins were subjected to DEAE cellulose column chromatography.

About 1.2 mg of protein was loaded onto a DEAE cellulose column (10 x 1 cm) pre – equilibrated with 0.05M Tris – HCl buffer. Elution was carried out first with l in buffer. Twenty `two` ml fractions were collected in each step of elution and the elution of the protein was monitored in a UVIKON 930 spectrophotometer at 220 nm.

Three major proteins were eluted in each buffer, and the protein fractions were designated as fraction I (buffer eluant), fraction II (eluant of 0.05 M NaCl in buffer) and fraction III (0.3 M NaCl in buffer). The thiol content of the isolated COM protein fractions was estimated by the method Ellman 1959(4).Oxalate binding assay was carried out using the method of Seethalaksahmi *et al.* (1986). (5)

Specific binding was calculated by subtracting non- specific binding from total binding and expressed as pmoles of oxalate bound / mg protein.

Statistical evaluation

Data are presented as mean S.D. Statistical analysis was carried out using ANOVA SPSS for windows,Release9.05.

RESULTS

Among the COM binding proteins, control fraction I had the maximum – SH content, which is followed by fraction II. The -SH content of all the three COM binding protein fractions were significantly decreased in KSP [Table 3], when compared to that of control [p<0.001].VitaminE supplementation restored the - SH content in all three fractions by nine months. The oxalate binding activities of the COM binding proteins were assayed [F I and F II – pH 7.4; F III – pH 4. All the three fractions had oxalate binding activity and the results are shown in Table 4. Maximal oxalate binding activity was associated with F II. Vitamin E supplementation to these renal stone formers normalized the oxalate binding activities of all the three fractions.

Table 1: Effect of vitamin E treatment on the total COM thiol content of COM binding proteins

Particulars	F I	F II	F III
Control (GP I)	4.8 0.05 ^{b***}	4.0 0.04 ^{b***}	3.6 0.03 ^{b***}
KSP (GP IIa)	3.6 0.04 ^{a***}	2.9 0.03 ^{a***}	2.9 0.02 ^{a***}
Treated			
3rd month (GP IIb)	3.9 0.03 ^{a***bNS}	3.2 0.03 ^{a***b**}	3.2 0.03 ^{a**b**}
6th month (GP IIc)	4.5 0.05 ^{aNSb**}	3.5 0.03 ^{a**b***}	3.4 0.03 ^{aNSb**}
9th month (GP IId)	4.7 0.04 ^{aNSb***}	3.7 0.03 ^{aNSb***}	3.5 0.03 ^{aNSb***}

Values are mean S.D. for 10 experiments expressed as µ g / mg protein.

Values are statistically different compared to a – control; b –when *** $p < 0.001$; ** $p < 0.01$; NS – Non - Significant.

Table 2: Oxalate binding activity of urinary COM binding proteins of control and various groups

Particulars	Fraction I (7.4)	Fraction II (7.4)	Fraction III (4.5)
Control (GP I)	252.6 20.7 ^{b***}	285.6 22.7 ^{b***}	255.1 20.3 ^{b***}
KSP (GP IIa)	334.2 33.1 ^{a***}	230.4 16.1 ^{a***}	197.7 13.7 ^{a***}

Treated			
3rd month (GP IIb)	282.7 17.4 ^{a*b**}	262.0 13.8 ^{a***b***}	225.1 12.8 ^{a**b**}
6th month (GP IIc)	279.0 18.6 ^{a*b**}	270.4 16.4 ^{a**b***}	235.3 17.4 ^{a*b**}
9th month (GP IId)	258.5 21.0 ^{aNSb***}	273.8 19.2 ^{a*b***}	247.5 20.1 ^{aNSb***}

Values are expressed as pmoles/mg protein, values were significantly different from a=control, b=KSP when compared with the treated groups. Symbols represent statistical significance *** $p<0.001$,** $p<0.01$, *$p<0.05$, NS - Non - Significant

DiscussionAmong the COM binding proteins, control fraction I had the maximum – SH content, which is followed by fraction II. The -SH content of all the three COM binding protein fractions were significantly decreased in KSP [Table 3], when compared to that of control [$p<0.001$]. Vitamin E supplementation restored the - SH content in all three fractions by nine months

Depletion in – SH contents have been reported in peroxidised tissue fractions already in our lab (6). Upon supplementation of vitamin E, the depletion of –SH content is minimized and there by suggesting that vitamin E prevented the peroxidation.

Fraction I and II exhibit oxalate binding activity at pH 7.4, which is nearer to physiological urinary pH, suggesting that they can bind urinary oxalate leading to biological consequences that are involved in lithogenesis. Fraction III has oxalate binding activity only at pH 4.5. Oxalate binding histones (H1) exhibits binding activity at pH 4.2 (7).In our laboratory, we have isolated three major calcium oxalate binding protein from human kidneys using the same methodology (8) and the corresponding molecular weights of the DEAE cellulose fractions were found to be 45 kDa, 20 kDa and 23 kDa. Fraction III derived from human kidney is found to be basic in nature and has oxalate binding activity at pH 4.5 (9).

A significant high oxalate binding activity is observed for hyperoxaluric fractions. The total urinary calcium binding protein activity is significantly greater in the active calcium oxalate stone formers when compared with either inactive calcium oxalate stone formers or non-stone forming controls.

CONCLUSION

The thiol content of all the three fractions was decreased in stone formers. Supplementation of vitamin E restored the SH contents. Fraction I and II had oxalate binding activities at pH 7.4, while F III had oxalate binding activity at pH 4.5 only. Non – stone formers showed higher oxalate binding activity for F II (285.6 pmoles / mg protein) whereas stone formers showed higher oxalate binding activity for F I (334.2 pmoles / mg protein). These changes in oxalate binding protein are reverted back to control levels in treated patients.

F I behaved as promoter of calcium oxalate crystallization, whereas FII and F III was nearly 2.2 fold increased in stone formers and also there was significant loss in the inhibitory activity of F II and III. These altered kinetics were moderated upon supplementation of vitamin E.

REFERENCES

1) Daudon, M., Bader, C.A. and Junger, P. (1993) Urinary calculi: review of classification methods and correlations with etiology. Scan. Microsc., 7: 1081 – 1106.
2) Hermsdorf, C.L. and Bronner, F. (1975) Vitamin D – dependent Calcium binding protein from rat kidney. Biochem. Biophys. Acta .,379: 553.
3) Kalaiselvi, P., Udayapriya, K.L. and Selvam, R. (1999) Uric acid – binding proteins in calcium oxalate stone formers and their effect on calcium oxalate crystallization. Br. J. Urol., 83 (9): 919 – 923.
4) Ellmann, GL. (1959) Tissue sulfhydryl groups. Arch . Biochem. Biophys., 82: 70 –77.
5) Seethalakshmi Lakshmanan, Selvam, R., Mahle, C.J. and Menon, M. (1986) Binding of oxalate to mitochondrial inner membranes of rat and human kidney.
6) Selvam, R. and Kalaiselvi, P. (2001) Studies on calcium oxalate binding proteins: Effect of Lipid peroxidation. Nephron., 88: 163.
7) Selvam, R. and Prasannalakshmi V. (1996) Occurence of histone – related oxalate binding in rat liver nucleus. Molecular and Cellular Biochemistry, 156 : 93-100.
8) Selvam, R. and Kalaiselvi, P. (2000) A novel basic protein from human kidney which inhibits calcium oxalate crystal growth. Br. J. Urol., 86: 7-13.
9) Kalaiselvi, P. and Selvam, R. (2001) Effect of experimental hyperoxaluria on renal calcium oxalate monohydrate binding proteins in the rat. Br. J. Urol., 87: 110- 116 J. Urol., 135: 862 – 865.