Cyclosporine-A Induced Nephrotoxicity is Associated with Decreased Renal Bmp-7 Expressıon in Rats

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Introduction

The bone morphogenetic proteins are a subgroup of the transforming growth factor-β (TGF-β) superfamily and is a key morphogenic signal for renal development (1). Its role has been investigated in a number of animal models of renal disease, including acute ischemic injury, tubulointerstitial fibrosis, diabetic nephropathy and lupus nephritis (2). BMP-7 administration, in the unilateral ureteral obstruction model in rodents which results in tubulointerstitial fibrosis leading to progressive renal damage, prevented interstitial inflammation and fibrogenesis (3).

Cyclosporine A(CsA) has considerably improved the survival of solid organ transplants and has been widely used since its first introduction in 1976. Chronic cyclosporine toxicity has been described in both renal and non-renal allograft recipients as well as in patients being treated with CsA for autoimmune diseases. Chronic cyclosporine toxicity may progress to an irreversible renal lesion characterized by interstitial fibrosis, tubular atrophy and the pathognomonic lesion of afferent arteriolar hyalynosis (5,6). The possible pathogenetic effect of BMP-7 depletion has not been studied for calcineurin induced nephrotoxicity. The aim of the present study is to investigate BMP-7 expression in a rat model of chronic cyclosporine (CycA) toxicity compared to healthy controls.

Methods

Chronic CsA nephrotoxicity model in the rat produced by the administration of CsA was used in this study. Three groups of eight male Wistar rats weighing 180-200g and fed on low sodium diet were included in the study. Group 1(H) animals (n=8) did not receive any treatment through the study period and were taken as healthy controls while Group 2 animals (n=8) were treated with a daily dose of CsA 15mg/kg intraperitoneally for 8 weeks. Group 3 (CsA+Q) animals (n=8) were treated with the same daily dose of CsA for 8 weeks plus quinapril (Aquitel, Pfizer, Turkey) 10 mg/kg/d in drinking water. During the experiments, animals were housed one per cage, maintained under controlled environmental conditions (12-h light/dark cycle, temperature 21°C). All animal procedures were in accordance with the declaration of Helsinki and guide for the care and use of laboratory animals. Study protocol was approved by local ethic committee of Marmara University.

Serum creatinine concentration were measured spectrophotometricaly while erythrocyte CycA levels were determined by flourecent polarization immune assay.

At the end of the study period the study rats were anesthesized by ether aneasthesia and sacrificed. Kidneys were excised and one was fixed in %0.4 parafolmaldehyde for light microscopic studies.

After an appropriate dehydration, kidney slices were embedded in paraffin, sectioned at 4□, and heamatoxylene eosine and trichromic stains were performed. The renal tissues were examined by light microscopy for the findings of CycA toxicity assessed by the presence of tubulointerstitial damage and arteriolar hyalinosis. Arteriolopathy percentage was determined by ocular micrometry. The results are expressed as the percentage of affected arterioles over total number of arterioles. The degree of tubulointerstitial fibrosis was evaluated semiquantitatively in trichrome stained specimens. The renal tissues were also immunohistochemically stained with BMP-7. BMP-7 expression was then semiquantitatively scored after the standard immunohistochemical staining (Santa Cruz-Biotechnology-SC-6899). The histological analysis was performed by two pathologists blind to each other and to the groups at which the renal tissues belonged.

Statistical analysis were performed with an IBM compatible PC using Graphpad Instat III program. Kruskal Wallis ANOVA was used for comparisons of the groups. Spearman Rank test was used for the correlation analysis. Data are expressed as mean ± SEM.

Results

Two rats from group 2 and from group3 died during the treatment period, while all others well tolerated the treatment through the study. Mean serum CsA levels were 1982ng/ ml and 1968ng/ml for CsA and CsA+Q group respectively . Mean serum creatinine levels were 0.8 + 0.2 mg/dl, 1.6 + 0.8 mg/dl and 1.4 + 0.8 mg/dl in group 1(H), 2(CsA) and 3(CsA+Q) respectively at the end of the treatment period. The difference was statistically significant between treatment groups and healthy controls ($p<0.005$) while it remained non-significant between the treatment groups.

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Table 1: Tubular and glomerular BMP-7 expression p<0.0005 for group CsA vs H; p<0.05 for group CsA+Q vs CsA

Study rats	Group H	Group CsA	Group CsA + Q
R1	+++	++	++
R2	+++	+	++
R3	+++	+	++
R4	+++	+	++
R5	+++	++	+
R6	+++	+	+

Histological studies revealed interstitial fibrosis at a degree of 25 % in 5 and 50% in one of the renal tissues in CsA treated group while tubular atrophy was found in all the studied rats of the latter group. Arteriolar lesions were observed in all CsA treated rats. The affected arterioles demonstrated an enlargement of the smooth muscle cells resulting in a partial or total narrowing of the arteriolar lumen. The affected cell cytoplasm showed eosinophylic, homogeneous or granular degeneration interpreted as arteriolar hyalyinosis. Interstitial fibrosis was not observed in healthy controls. Interstitial fibrosis in Group 3 animals (CsA+Q) was at a degree 25% in all animals

Afferent arteriolopathy was observed in 30% of the total evaluated afferent arterioles in CsA only treated group (group2) with early hyalonisos in 4 and late hyalinosis in 2 while only in 1.8% of the healthy controls. Arteriolar lesions were present in 20% of the total evaluated afferent arterioles in CsA+Q group. The difference between the two treatment groups failed to reach significance.

Immunohistochemical staining revealed significantly decreased BMP-7 expression in CsA treated animals as compared with controls (P<0.0005). There was also significantly higher BMP-7 expression in CsA+Q group compared to CsA only group (P<0.05) (Table 1). Within the CycA treated group BMP-7 expression seems to be negatively correlated with interstitial fibrosis.

Discussion

Our study is the first addressing the role of BMP-7 in chronic CsA toxicity. In this rat model of CsA induced nephrotoxicity characteristic histologic changes were associated with altered expression of BMP-7. BMP-7 has an essential role throughout the development of the kidney. Recent data lead to the speculation that BMP-7 deficiency may be important in acute and progressive renal disease. Following its beneficial effect on acute ischemic renal injury, BMP-7 has also been investigated for its role in the treatment of tubulointerstitial fibrosis(3,4).In a rat model of unilateral ureteral obstruction, Hruska et al have reported that BMP-7 administration protected against tubulointerstitial fibrosis when given in a prevention protocol. Although the role of BMP-7 in renal injury is largely unknown, this protein may have a cytoprotective effect or may be regulating chemotactic cytokines involved in monocyte infiltration associated with diverse renal disease. Tubulointerstitial fibrosis is a disease process implicated in a variety of progressive renal diseases including chronic CsA nephrotoxicity encountered mainly in transplanted subjects. Chronic CsA nephrotoxicity is the major longterm drawback of this potent immunosuppressive agent.

The group receiving quinapril, an angiotensin converting enzyme (ACE) inhibitor, in addition to CsA exhibited a higher BMP-7 expression when compared to the group receiving CsA only. This finding suggests that ACE inhibition may have an influence on the upregulation of BMP-7 and support the proposed antifibrotic effect of the ACE inhibitors. However, BMP-7 expression was still lower in CsA+Q group than the healthy controls.

In conclusion the results of our study suggest a down regulation of BMP-7 expression in this rat model of cyclosporine toxicity implicating its potential utility in the prevention of chronic calcineurin toxicity. Furthermore, the decreased BMP-7 expression in CsA induced nephrotoxicity seems to be favorably affected by ACE inhibition. Further studies are needed to confirm these findings and to investigate its potential use for the treatment of cyclosporine toxicity.

References

1. Lund RJ, Davies MR, Hruska K: Bone Morphogenetic protein-7: an anti-fibrotic morphogenetic protein with therapeutic importance in renal disease. Curr Opin Nephrol Hypertens 11:31-36; 2002
2. Klahr S, Morrissey J, Hruska K, Wang S, Chen Q:New approaches to delay the progression of chronic renal failure. Kidney Int 61:Suppl 80: S23-S26; 2002.
3. Hruska KA. Treatment of chronic tubulointerstitial disease: A new concept. Kidney Int 61: 1911-1922; 2002.
4. Bone Morphogenetic Protein-7 improves renal fibrosis and accelerates the return of renal function. Morissey J, Hruska K, Guo G, Wang S, Chen Q, Klahr S. J Am Soc Nephrol 2002; 13:S14-S21.
5. Young BA, Burdmann EA, Johnson RJ, Andoh T, Bennett WM, Couser WG, Alpers CE. Cyclosporine A induced arteriolopathy in a rat model of chronic cyclosporine nephropathy. Kidney International 48:431-438;1995.
6. Young BA, Burdmann EA, Johnson RJ, Alpers CE,Giachelli CM, Eng E, Andoh T, Bennett WM, Couser WG. Cellular proliferation and macrophage influx precede interstitial fibrosis in cyclosporine nephrotoxicity 48: 439-448; 1995.