BMC Nephrology

Research article

Angiotensin converting enzyme inhibitor therapy in children with Alport syndrome: effect on urinary albumin, TGF-β, and nitr ite excretion

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Published: 14 February 2002

BMC Nephrology 2002, **3**:2

Received: 7 December 2001
Accepted: 14 February 2002

This article is available from: http://www.biomedcentral.com/1471-2369/3/2

Keywords: Alport syndrome, angiotensin converting enzyme inhibitors, transforming growth factor-β, urinary nitrite excretion

Abstract

Background: Angiotensin converting enzyme inhibitors are routinely prescribed to patients with chronic kidney disease because of their known renoprotective effects. We evaluated the effect of short-term therapy with the angiotensin converting enzyme inhibitor, enalapril, in early Alport syndrome, defined as disease duration less than 10 years and a normal glomerular filtration rate.

Methods: 11 children with early Alport syndrome were investigated. Two consecutive early morning urine specimens were collected at the start of the study for measurement of urinary creatinine, total protein, albumin, TGF-β, and nitrite excretion. Patients were treated with enalapril, ≅ 0.2 mg/kg/day, once a day for 14 days. Two early morning urine specimens were collected on days 13 and 14 of enalapril treatment and two weeks later for measurement of urinary creatinine, total protein, albumin, TGF-β, and nitrite excretion.

Results: Prior to treatment, urinary excretion of transforming growth factor-β and nitrite, the major metabolite of nitric oxide, was within normal limits in all patients. Administration of enalapril for 2 weeks did not alter urinary albumin, transforming growth factor-β, or nitrite excretion.

Conclusion: These findings suggest that early Alport syndrome represents a disease involving exclusively intrinsic glomerular barrier dysfunction. At this stage of the illness, there is no evidence of angiotensin II-mediated proteinuria or increased production of transforming growth factor-β and, therefore, routine treatment with an angiotensin converting enzyme inhibitor may not be warranted.

Background

Alport syndrome (AS) is a glomerulopathy caused by genetic mutations in type IV collagen, the main collagenous constituent of the glomerular basement membrane (GBM) [1]. The predominant form of AS is inherited as an X-linked dominant disease. Hematuria is the initial finding and proteinuria develops later secondary to the defective composition and ultrastructure of the GBM [1]. Proteinuria increases with age and is a useful marker of disease progression. End stage renal disease (ESRD) eventually ensues in affected males, while the disease course is more benign in females. There is no specific therapy for AS and renal transplantation remains the definitive treatment [1].

Transforming growth factor-β (TGF-β) and nitric oxide (NO) are important mediators of renal disease progression. TGF-β is a fibrogenic cytokine involved in normal wound healing and pathological fibrosis (). Intra-renal production of TGF-β is enhanced in patients with IgA nephropathy, diabetic nephropathy, lupus nephritis, and focal segmental glomerulosclerosis (FSGS) when they develop glomerular scarring [3]. The factors that control TGF-β overexpression in these renal diseases are not well understood. NO is a short-acting signaling molecule that is involved in the regulation of glomerular hemodynamics, mesangial proliferation, and net mesangial matrix production [4]. Diminished renal production in chronic renal disease may contribute to kidney fibrosis [5]. The role of TGF-β and NO in promoting progressive disease in AS is not known.

Treatment with angiotensin converting enzyme inhibitors (ACEI) is renoprotective in virtually all proteinuric forms of kidney disease [6]. By reducing intraglomerular hypertension, ACEI prevent glomerular hyperfiltration. However, because angiotensin II is a potent inducer of TGF-β release, ACEI may also diminish renal fibrosis by a non-hemodynamic mechanism [7,8]. Both ACEI and NO retard the progression to kidney failure and their renoprotective effects correlate with suppression of TGF-β [9–12].

In view of these observations, we performed the following study to determine urinary protein, TGF-β, and nitrite (the stable metabolite of NO) excretion in children with early AS and to evaluate the impact of short-term ACEI therapy on these measurements.

Materials and Methods

Patients:

11 children (5M:6F), mean age 8.6 ± 1.1 years, with renal early biopsy-proven AS were included in this study. The duration of disease from the date of diagnostic biopsy until participation in this study was 56 ± 12 months. The estimated glomerular filtration rate (GFR) was calculated using the age and sex-appropriate length-serum creatinine formulas [13,14]. Normal levels of blood pressure were defined based upon the updated Task Force Report on Blood Pressure Control in Children and Adolescents [15].

Clinical protocol:

Two consecutive early morning urine specimens were collected at the start of the study for measurement of urinary creatinine, albumin, TGF-β, and nitrite excretion. Patients were then given enalapril (EN), ≅ 0.2 mg/kg/day, once a day for 14 days. The daily dose of enalapril was between 5–10 mg. The amount of drug and duration of treatment were the same as in a previous evaluation of ACEI therapy in sickle cell nephropathy [16]. Two early morning urine specimens were collected on days 13 and 14 of EN treatment. Administration of the ACEI was then stopped and two early morning urine specimens were collected again two weeks later. This study was approved by the Institutional Review Board of the Schneider Children's Hospital of the North Shore-Long Island Jewish Health System and informed consent was signed prior to patient enrollment.

Analytical methods:

Urine creatinine was measured using standard methods. Urinary albumin and TGF-β were determined using commercially available EIA kits (R & D Systems, Minneapolis, MN). Urinary albumin excretion was expressed as the urine albumin: creatinine ratio (UAC) (mg/mg). The Greiss reaction was used to measure urinary nitrite excretion, which was expressed as nmol/mg creatinine.

Statistical analysis:

Data are presented as mean ± SE. Differences between groups were analyzed using the ANOVA and t-test and results were considered significant if $P < 0.05$.

Results

In the 11 patients who enrolled in this clinical study, the estimated GFR was 146 ± 14 ml/min/1.73 m^2 . Blood was not drawn at the end of the 14-day EN treatment period and, as a consequence, the effect of ACEl therapy on GFR could not be determined. All of the children had a normal blood pressure without antihypertensive medications. Prior to EN treatment, the mean UAC was 1.2 ± 0.9 (range: 0.01–9.6). In addition, the level of TGF-β in the urine was 60 ± 11 pg/mg creatinine, within the range observed in healthy subjects (50–300 pg/mg creatinine) [17]. Finally, the baseline urinary nitrite excretion was 1466 ± 330 *versus* 836 ± 274 nmol/mg creatinine in normal control children ($P = 0.16$).

At the end of two weeks of EN therapy, UAC was 130 ± 33% of pre-treatment levels, and remained elevated 14 days after treatment was discontinued, namely 134 ± 31% of the baseline value. A decline in UAC >33% was ob-

served in only 2 patients, both of whose initial urine albumin concentration was >1000 mg/L (the urinary albumin: creatinine ratio (mg/mg) was 1.4 and 9.6 in these 2 children). In the urine samples collected after two weeks of ACEI therapy and after withdrawing EN two weeks later, the urinary TGF-β excretion was 55 ± 11 and 57 ± 10 pg/mg creatinine, respectively. Compared to pre-treatment levels, urinary TGF-β levels were 110 ± 23 and 112 ± 30%, respectively, of the baseline value at the completion of the 2-week course of ACEI therapy and 2 weeks after discontinuation of the medication ($P > 0.4$). Similarly, short-term ACEI therapy had no effect on urinary nitrite excretion, 92 ± 20 and 84 ± 7% of baseline level, after 2 wk of EN therapy, and 2 wk after discontinuing the ACEI, respectively ($P > 0.3$). The changes in urinary TGF-β and nitrite excretion were not different in the two patients with significant albuminuria compared to the other 9 children.

All of the urinary measurements, i.e., albuminuria, TGF-β, and nitrite excretion, were comparable in boys and girls at baseline and in response to ACEI therapy. Although there was no correlation between the effect of EN therapy on albuminuria and the change in urinary TGF-β or nitrite excretion, the later two variables were significantly related to one another ($P < 0.04$).

Discussion

The children with biopsy-proven AS who were enrolled in this study had early disease, based upon a normal GFR, normal blood pressure, and sub-nephrotic range proteinuria. The urinary abnormalities observed in these patients were probably a consequence of genetic mutations in the α5 chains of type IV collagen and defective GBM ultrastructure [1]. Although the long-term risk of disease progression differs in male *versus* female patients, based on the similar level of urinary protein excretion, it is likely that at the time of these investigations glomerular barrier function and the mediators of proteinuria were similar in all of the study participants.

In other progressive glomerulopathies, alterations in TGF-β and NO have been implicated in the steady decline in renal function, glomerulosclerosis, and tubulointerstitial fibrosis. The source of the fibrogenic cytokines and vasoactive mediators varies in different disease entities. For example, in IgA nephropathy, immune complex nephritis, and FSGS increased intra-renal levels of TGF-β and NO are the consequence of release by resident and/or infiltrating immunoeffector cells [3]. In contrast, in diabetic nephropathy, metabolic disturbances, namely hyperglycemia, directly modulate TGF-β and NO synthesis by glomerular mesangial and renal tubular epithelial cells [3,7]. In diabetic nephropathy, alterations in intra-renal TGF-β production have been assessed by measuring urinary excretion of this cytokine [17].

Measurements of urinary nitrite excretion are less interpretable than determinations of urinary nitrite + nitrate excretion because a variable portion (50–90%) of NO-related end products in the urine may represent nitrate [18]. However, nitrate excretion is primarily dependent on dietary intake and there is a close correlation between urinary nitrite and nitrate excretion in healthy subjects [18]. Therefore, because this was a short-term study and patients did not modify their diet for the duration of the 4-week experimental period, it is likely that the urinary nitrite excretion accurately reflects renal NO synthesis. The small non-significant decrease in urinary nitrite excretion after ACEI therapy, despite the known effect of these drugs to stimulate endothelial NO release, may be the consequence of a decline in GFR. A blood sample was not obtained at the conclusion of ACEI treatment to enable measurement of renal function. However, most pediatric patients with a GFR in the range noted for the children in this study do not develop a decline in renal function in response to EN therapy.

Our data indicate that in early AS, hematuria and sub-nephrotic range proteinuria are exclusively the result of intrinsic glomerular barrier dysfunction and are not the consequence of acutely reversible alterations in glomerular hemodynamics. In contrast to children with type I diabetes mellitus [17], there is no increase in renal TGF-β or NO production at this stage of the disease. The clinical data are in agreement with recent findings in a transgenic model of autosomal recessive Alport syndrome in which TGF-β was only involved in the late progression of the nephropathy [19,20]. Although male patients with AS are at greater risk than females of developing progressive kidney failure, our findings suggest that differences in renal production of TGF-β in the early phase of the disease cannot explain the impact of gender on prognosis. Although higher levels of proteinuria may stimulate release of these and other inflammatory mediators, this pathogenetic mechanism is not activated in early AS.

In virtually all forms of glomerular disease, administration of ACEI to patients with nephrotic range proteinuria results in a significant and reproducible reduction in urinary protein excretion and presumably an improvement in the long-term prognosis [6]. However, early in the course of AS, when our patients had sub-nephrotic range proteinuria there was no evidence of an angiotensin II-sensitive alteration in glomerular hemodynamics that manifested as a decrease in albuminuria. The more pronounced antiproteinuric response in the 2 children whose initial urine albumin concentration exceeded 1000 mg/L is consistent with a previous report of enalapril use in pediatric patients with AS [21].

It is important to note that our protocol to determine the effect of short-term administration of ACEI on urinary protein excretion has been effective in other forms of glomerular disease in which hemodynamic alterations have been linked to perturbations in glomerular permselectivity, namely sickle cell nephropathy [16]. We cannot exclude the possibility that once-a-day administration of EN was ineffective because of different pharmacokinetics and a shorter drug half-life in children. The response to short-term ACEI was significantly lower in our patients with AS (2/11 children had >33% reduction in albuminuria) *versus* published reports in adults with sickle cell nephropathy (10/10 had a comparable decrease in albuminuria) ($P < 0.0005$) [16]. The 95% confidence interval on this difference indicates that at most 40% of children with AS are likely to demonstrate a meaningful reduction in albuminuria following 2 weeks of EN therapy. Thus, despite the small sample size in this study, it is reasonable to conclude that ACEI treatment will be ineffective in lowering urinary protein excretion in children with early AS.

Whether long-term ACEI therapy is effective in patients with AS who have developed heavy proteinuria is uncertain. Additional studies will be needed to determine whether ACEI treatment at later stages of the disease reduces proteinuria, precisely when in the disease course ACEI responsiveness supervenes, and whether this change is paralleled by alterations in urinary TGF-β and nitrite excretion. Experimental protocols designed to evaluate the impact of ACEI therapy in transgenic models of AS will help clarify the role of this treatment at various stages in the course of the disease.

Conclusion

Our results suggest that early AS represents an isolated defect in GBM composition and ultrastructure resulting in a pure glomerular protein leak. At this stage of the disease, there is no stimulus to increase TGF-β or NO production nor is there evidence of an angiotensin II-sensitive alteration in glomerular hemodynamics. Short-term administration of an ACEI to patients with early AS does not cause a reversible decrease in proteinuria. We speculate that with longer duration of disease and increasing urinary protein excretion, the pathophysiology of AS becomes similar to other glomerulopathies with involvement of altered glomerular hemodynamics, inflammatory mediators, chemokines, and fibrogenic cytokines. At this advanced stage, aggressive ACEI therapy may have a renoprotective role. Further studies are needed to determine the exact time point in the course of AS when administration of an ACEI is a useful intervention to effectively lower urinary protein excretion and stabilize renal function.

Competing Interests

None

Acknowledgement

This work was supported in part by a grant from Merck Inc. (West Point, PA).

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