Pediatr Nephrol (2000) 14:1088–1091 

ORIGINAL ARTICLE

**Angela Delucchi · Francisco Cano**
**Eugenio Rodriguez · Eduardo Wolff**
**Ximena Gonzalez · Miguel A. Cumsille**

# Enalapril and prednisone in children with nephrotic-range proteinuria

Received: 6 October 1998 / Revised: 7 October 1999 / Accepted: 7 October 1999

**Abstract** The effect of enalapril and low prednisone doses on the urinary protein electrophoretic pattern was studied in 13 pediatric patients with glomerular diseases and steroid-resistant nephrotic syndrome. Enalapril was administred at doses of 0.2–0.6 mg/kg per day for 24–84 months, and prednisone was introduced 2 months later in 11 patients at doses of 30 mg/m$^2$ on alternate days. The urine protein electrophoretic pattern showed a reduction of 80% and 70% in the total protein and albumin, respectively, after enalapril. Total urinary protein decreased from 5.46 to 1.1 g/m$^2$ per day ($P$<0.001). A marked change from a pattern of non-selective urinary protein loss to an albumin-selective proteinuria was observed. Mean total plasma proteins increased from 4.7 to 5.43 g/dl ($P$<0.001). Four patients became free of proteinuria 24 months after enalapril was started, but only 2 remained free of proteinuria at 48 months of follow-up. The other 11 patients had persistent albuminuria of between 0.5 and 2.6 g/m$^2$ per day with a selective urinary electrophoretic pattern. No additional decrease was observed after steroids were introduced. A clinical improvement in edema was observed in all children. Three patients developed transient acute renal failure, during the course of an infectious disease; 2 developed peritonitis and 1 pneumopathy. In these patients withdrawal of enalapril was necessary until a complete recovery of renal function was observed. Four patients were hypertensive on admission, achieving normal blood pressure 1 month after enalapril was started. No episodes of systemic arterial hypotension were seen. Creatinine clearance and serum potassium showed no statistically significant change.

**Key words** Enalapril · Nephrotic-range proteinuria · Electrophoretic urinary pattern

A. Delucchi · F. Cano · E. Rodriguez · E. Wolff
Division of Pediatric Nephrology, Hospital Luis Calvo Mackenna, Universidad de Chile, Santiago, Chile

X. Gonzalez
Biochemistry Laboratory, Hospital Luis Calvo Mackenna, Universidad de Chile, Santiago, Chile

M.A. Cumsille
Department of Biostatistics, Hospital Luis Calvo Mackenna, Universidad de Chile, Santiago, Chile

A. Delucchi (✉)
Augusto Ovalle no. 1330-901, Santiago, Chile
e-mail: delucchi@cmet.net
Fax: +56-2-3415178

## Introduction

Despite a better understanding of etiopathogenic mechanisms, steroid-resistant nephrotic syndrome (SRNS) still represents a challenge for the nephrologist, not only in treatment of the glomerulopathy but also in the remission of proteinuria, which is strongly associated with edema and complications such as sepsis, thromboembolism, malnutrition, and progression of renal damage. In 90% of cases, idiopathic nephrotic syndrome in childhood corresponds to a minimal change disease (MCD), and of these 85% benefit from steroid treatment. The other, less-frequent causes of SRNS are focal segmental glomerulosclerosis (FSGS) and membranoproliferative glomerulonephritis (MPGN), with more than 50% of patients progressing to end-stage renal failure within 2–10 years. Despite this, these diseases have a variable progression in their nephrotic characteristics [1, 2].

The use of immunosuppressive therapy, such as oral or intravenous cyclophosphamide, azathioprine, or chlorambucil, has had disappointing results and undesirable side effects [3, 4]. In pediatric patients cyclosporine has been used successfully in steroid-dependent nephrotic syndrome. However the majority of these patients became cyclosporine dependent with a high risk of nephrotoxicity, as shown by renal biopsies [5]. Recently, Niaudet et al. [6] reported the use of intravenous cyclosporine in recurrent SRNS, but the benefit of cyclosporine in these patients was still debatable. Recently, angiotensin-converting enzyme inhibitors (ACEI) have been proposed as a treatment for steroid non-responding nephrotic syndrome, showing a variable decline of pro-

teinuria. The antiproteinuric effect of ACEI has been ascribed to various factors, including a decrease in the glomerular capillary plasma flow rate, a decrease in transcapillary hydraulic pressure, or a change in the permselectivity of the glomerular filtering membrane at the level of the glomerular capillary wall [7–10].

New advances in electrophoresis for the separation of urinary proteins according to molecular size have allowed different patterns of proteinuria in renal diseases to be distinguished. The purpose of this study was to evaluate the effect of enalapril on the plasma and urine electrophoretic patterns.

## Patients and methods

### Study population

The study population included 13 patients with SRNS, all secondary to glomerular diseases (10 boys, aged 1.8–12 years, mean 8 years) were treated for 24–84 months (mean 48 months). Renal biopsies showed: FSGS (*n*=4), type I MPGN (*n*=3), crescentic glomerulonephritis (*n*=3), 2 cases secondary to Henoch-Schonlein disease and the other to post-streptococcal glomerulonephritis, MCD with IgM deposition in 2 patients and diffuse mesangial sclerosis in the youngest patient.

All showed persistent nephrotic-range proteinuria above 40 mg/m$^2$ per hour, and had received prednisone (60 mg/m$^2$ per day) for at least 8 weeks, according to standard recommendations, without response. In addition, 8 patients received cyclophosphamide therapy for 2 months (2 mg/kg per day) and 5 patients were treated with methylprednisolone bolus 1 g/m$^2$ (3 doses), after standard oral prednisone treatment, but despite this no change was observed in proteinuria [11, 12].

### Urine collection, blood samples, and electrophoresis

Urine collection began after the first-morning voiding at 7 a.m. and included the first-morning urine at 7 a.m. the following day. Blood samples for serum creatinine and electrolytes were taken in the morning while fasting. Creatinine clearance was estimated from the serum creatinine using the Schwartz formula.

Electrophoresis is a reliable method for detecting serum and urinary protein changes associated with certain broad disease categories. In our study electrophoresis was performed at pH 8.6 on agarose gel, which separates serum and urinary proteins into five distinct and well-defined bands. These protein bands were identified in order of decreasing electrophoretic mobility as albumin, alpha$_1$-globulin, alpha$_2$-globulin, beta globulin, and gamma globulin. Following electrophoresis, the separated protein bands were visualized by staining with amido black 10 B. The dried agarose film is durable and shows little or no deterioration upon storage. Quantification was easily accomplished with densitometry due to the clarity of the finished electrophoretogram [13, 14].

Urine and blood samples were obtained at the beginning of the treatment, twice a month for 2 months, and once a month thereafter, until the proteinuria had decreased by 50%, and once every 3 months until the end of the study.

### Treatment

Enalapril was started at 0.2 mg/kg per day (maximum 30 mg/day) and increased by 0.1 mg/kg per day each month until a 50% decrease in basal proteinuria was reached. Prednisone was introduced 2 months later in 11 patients at doses of 30 mg/m$^2$ on alternate days with the aim of further reducing proteinuria (Table 1).

### Statistical analysis

The data are presented using statistical analysis of median values and non-parametric Wilcoxon tests.

## Results

Four patients showed a complete remission of proteinuria 4–12 months after enalapril was started. The other patients showed an 80% and 70% decrease in both urinary total protein and albumin excretion, respectively. A clinical improvement in edema was observed in all children. Four patients who had arterial hypertension on admission achieved normal values of arterial pressure according to the Task Force Report [15] 1 month after enalapril was started. Mean arterial pressure was 91±10

**Table 1** Enalapril and prednisone in nephrotic-range proteinuria – follow-up of urinary protein (g/m$^2$ per day) (*DMS* diffuse mesangial sclerosis, *FSGS* focal segmental glomerulosclerosis, *MPGN* membranoproliferative glomerulonephritis, *CGN* crescentic glomerulonephritis, *MCD* minimal change disease)

| Underlying disease | Before treatment | After classical treatment | Before enalapril 0 months | After enalapril 2 months | Alternate-day prednisone | 6 months | 12 months | 24 months | 36 months | 48 months |
|---|---|---|---|---|---|---|---|---|---|---|
| DMS | 2.92 | 3.5 | 2.65 | 1.05 | 10 | 0.8 | 0.9 | 1.2 | 0.9 | 0.5 |
| FSGS | 5.2 | 4.98 | 5.46 | 1.25 | 15 | 1.2 | 0.8 | 1.8 | 2.0 | 2.2 |
| FSGS | 5.46 | 10 | 12.3 | 4.5 | 20 | 2.7 | 1.0 | 1.2 | 1.4 | 1.1 |
| FSGS | 8.6 | 7.5 | 5.2 | 1.9 | 25 | 1.8 | 1.8 | 1.9 | – | – |
| FSGS | 10.5 | 8.0 | 8.6 | 6.5 | 15 | 4.1 | 2.7 | 2.2 | 2.0 | 1.9 |
| MPGN | 4.79 | 5.6 | 4.7 | 1.7 | 10 | 1.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| MPGN | 4.0 | 6.0 | 4.2 | 1.52 | 25 | 1.6 | 1.0 | 1.2 | 1.4 | 1.2 |
| MPGN | 4.4 | 5.34 | 5.45 | 2.68 | 30 | 2.8 | 1.2 | 1.8 | 1.9 | 1.6 |
| CGN | 8.9 | 3.0 | 8.6 | 1.08 | – | 0.0 | 0.0 | 2.5[a] | 1.3 | 0.9 |
| CGN | 8.7 | 5.0 | 8.2 | 1.57 | 15 | 1.0 | 0.0 | 1.9[a] | 1.8 | 0.7 |
| CGN | 5.45 | 5.9 | 6.0 | 0.8 | 10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| MCD | 2.65 | 2.9 | 2.92 | 2.96 | 20 | 2.6 | 2.6 | 2.8 | – | – |
| MCD | 7.65 | 5.0 | 10.5 | 1.89 | 20 | 1.6 | 1.1 | 1.9 | 2.6 | 2.6 |

[a] Enalapril was restarted

**Table 2** Enalapril and prednisone in nephrotic-range proteinuria – serum and urine electrophoresis

| | Urinary protein ($g/m^2$ per day) | | | Serum protein (g/dl) | | |
|---|---|---|---|---|---|---|
| | Pre | Post | *P** | Pre | Post | *P** |
| Total protein | 5.46 | 1.1 | 0.001 | 4.7 | 5.43 | 0.001 |
| Albumin | 3.2 | 0.99 | 0.001 | 1.41 | 2.43 | 0.001 |
| $Alpha_1$-globulin | 0.4 | 0.0 | 0.02 | 0.25 | 0.30 | NS |
| $Alpha_2$-globulin | 0.57 | 0.0 | 0.001 | 1.55 | 1.14 | 0.01 |
| Beta globulin | 1.0 | 0.0 | 0.001 | 0.83 | 0.89 | NS |
| Gamma globulin | 0.15 | 0.0 | 0.009 | 0.50 | 0.65 | NS |

***** Values are median in urine and plasma

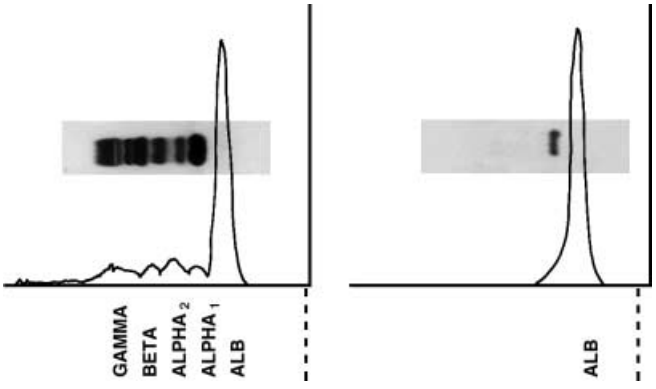


**Fig. 1** Electrophoretic pattern of urinary protein before and after enalapril. A significant change in the electrophoretic pattern was observed after the enalapril treatment. The non-selective proteinuria became an albumin-selective urinary protein loss (*GAMMA* gamma globulin, *ALPHA 2* $alpha_2$-globulin, *ALPHA 1* $alpha_1$-globulin, *ALB* albumin)

and 81±6.7 mmHg before and after enalapril ($P<0.02$). No additional significant change in blood pressure was observed, even in those patients with maximum enalapril doses. No episodes of systemic arterial hypotension were observed.

When the pattern of urinary proteins was analyzed, the initial non-selective pattern had changed to an albumin-selective urinary protein loss in all patients (Fig. 1). An average decrease from 5.46 to 1.1 $g/m^2$ per day ($P<0.001$) and 3.2 to 0.99 $g/m^2$ per day ($P<0.001$) was recorded for total urinary protein and albumin, respectively. Urinary globulins showed a significant decrease in all the urine protein fractions. The plasma electrophoretic protein pattern showed a significant increase in plasma levels of albumin and total protein (Table 2).

In 5 children creatinine clearance showed hyperfiltration secondary to nephrotic syndrome at the beginning of the study. After enalapril therapy was started the glomerular filtration rate was in the normal range in all patients and creatinine clearance showed no statistically significant change during the study. Mean creatinine clearance was 89±16.8 and 78±18.6 ml/min ($P<0.31$) before and after enalapril treatment. Three patients presented with mild hyperkalemia (mean 5.6 mEq/l), but the doses of enalapril were not changed. Of the 4 patients who became free of proteinuria, only 2, 1 with crescentic (poststreptococcal) glomerulonephritis and 1 with MPGN, remained free of proteinuria after 4 years of follow-up. The other 11 patients had persistent albuminuria of between 0.5 and 2.6 $g/m^2$ per day. In all, prednisone at doses of 30 $mg/m^2$ on alternate days was added to achieve better control of proteinuria. No additional decrease in proteinuria was observed in these patients after steroids were introduced (Table 1).

After 12 months of complete remission of proteinuria in 3 patients with crescentic glomerulonephritis, enalapril therapy was discontinued for 2 years. It was necessary to restart treatment in 2 patients because of recurrence of proteinuria, and a good response was obtained with a remission of proteinuria.

Few adverse side effects of enalapril therapy were observed in our patients. Three patients developed transient acute renal failure during the course of an infectious disease; 2 peritonitis and 1 pneumopathy. Enalapril treatment was interrupted until complete recovery of renal function was observed.

## Discussion

Since early clinical trials with diabetic patients and animal experiments, ACEI have proven to be useful in decreasing intraglomerular hypertension and progressive renal deterioration [16, 17]. Early trials in diabetic patients showed that ACEI reduced proteinuria without any further reduction in renal function [18]. Theoretical analyses of experimental data document that ACEI uniformly reduce the radius of all membrane pores in rats [19]. Morelli et al. [20] reported that, in humans with insulin-dependent diabetes, enalapril exerts an antiproteinuric effect by reducing the size of glomerular pores, thus enhancing the size-selective barrier.

Chronic renal disease evolves to end-stage renal failure through various stages, which include enhanced intraglomerular pressure and plasma protein ultrafiltration, produced at least in part by angiotensin II. ACEI reduce intracapillary pressure, leading to a recovery in glomerular size-selective function, which may account for their antiproteinuric and potential renoprotective effect [21, 22]. A selective decrease in intraglomerular transcapillary pressure has also been shown, not related to any change in systemic blood pressure, but mainly due to ACEI-induced efferent arteriolar dilation [23, 24].

Experiments in animals and humans have documented that inhibition of the renin-angiotensin system by ACEI reduces the urinary protein excretion rate and delays the development of renal injury. Angiotensin II induces changes in the urinary protein excretion rate and modulates glomerular capillary permselectivity in isolated perfused rat kidneys; these changes were completely prevented by angiotensin II receptor antagonist [25].

The first studies on the efficacy of ACEI treatment in children with SRNS appeared 10 years ago. Trachtman and Gauthier [8] reported a 50%–70% decrease in urinary protein loss, but no significant change was observed in blood pressure and creatinine clearance following this therapy. More recently, Proesmans et al. [9] studied the effect of enalapril in six pediatric patients with nephrotic proteinuria, and observed an important reduction of proteinuria in two patients and a moderate decrease in three others. However, therapy was accompanied by a fall in glomerular filtration in all subjects.

Dextran sieving studies showed that angiotensin II blockade reduced fractional clearance values for large neutral dextrans near to values observed in normal rats, but had no effect on the fractional clearance of dextran sulfate. These findings indicate that reducing angiotensin II activity improves size selectivity without affecting charge selectivity in injured remnant glomeruli [26].

Electrophoresis is a well-established technique for the analysis of proteinuria in renal diseases which allows the differentiation of urinary proteins according to their molecular weight. Many variations of polyacrylamide gel electrophoresis have been applied to routine clinical practice and numerous publications have classified proteinurias according to the different molecular weight patterns of urinary protein.

Our findings indicate that enalapril, an oral ACEI, can safely reduce urinary protein loss in children, changing a non-selective electrophoretic urinary protein pattern into a selective one. Otherwise, total protein and albumin plasma levels show a statistically significant increase, without any significant reduction in creatinine clearance. This kind of therapy seems to be useful and safe in the management of proteinuric nephrotic patients, allowing a better clinical outcome in children with SRNS.

## References


1. Cameron J (1988) The long-term outcome of glomerular disease. In: Schrier R (ed) Diseases of the kidney, 4th edn. Little Brown, Boston, pp 2127–2189
2. Bernard D (1988) External complications of the nephrotic syndrome. Kidney Int 33:1184–1202
3. Tarshish P, Tobin J, Bernstein J, Edelman C (1996) Cyclophosphamide does not benefit patients with focal segmental glomerulosclerosis. A report of the International Study of Kidney Disease in Children. Pediatr Nephrol 10:590–593
4. Remuzzi G, Schieppati A, Garattini S (1994) Treatment of idiopathic membranous glomerulopathy. Curr Opin Nephrol Hypertens 3:155–163
5. Tejani A, Butt K, Trachtmann H, Suthantiran M, Rosenthal C, Khawar M (1988) Ciclosporine A induced remission of relapsing nephrotic syndrome in children. Kidney Int 33:729–732
6. Niaudet P, Fuchshuber A, Gagnadoux M, Habib R, Broyer M (1997) Cyclosporine in the therapy of steroid-resistant idiopathic nephrotic syndrome. Kidney Int 58:85–90
7. Milliner DS, Morgenstern BZ (1991) Angiotensin converting enzyme inhibitors for reduction of proteinuria in children with steroid-resistant nephrotic syndrome. Pediatr Nephrol 5:587–590
8. Trachtman H, Gauthier B (1988) Effect of angiotensin converting enzyme inhibitor therapy on proteinuria in children with renal disease. J Pediatr 112:295–298
9. Proesmans W, Van Wambeke I, Van Dyck M (1996) Long-term therapy with enalapril in patients with nephrotic range proteinuria. Pediatr Nephrol 110:587–589
10. Jean L, Olson J, Heptinstall R (1988) Nonimmunologic mechanisms of glomerular injury. Lab Invest 59:564–577
11. International Study of Kidney Disease in Children (1978) The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. J Pediatr 98:561–572
12. Barrat TM, Clark G (1994) Minimal change nephrotic syndrome and focal segmental glomerulosclerosis. In: Holliday M, Barrat TM, Avner DE (eds) Pediatric nephrology. Williams and Wilkins, Baltimore, pp 781–787
13. Ehrich JH, Wurster U (1991) Differentiation of proteinurias with electrophoresis. Pediatr Nephrol 4:376–378
14. Brocklebank T, Cooper E, Richmond K (1991) Sodium dodecyl sulphate polyacrylamide gel electrophoresis patterns of proteinuria in various renal disease of childhood. Pediatr Nephrol 4:371–375
15. National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents (1996) Update on the 1997 Task Force report on high blood pressure in children and adolescent: a working group report from the National High Blood Pressure Education Program. Pediatrics 98:649–658
16. Anderson S, Meyer TW, Rennke HG, Brenner BM (1985) Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. J Clin Invest 76:612–619
17. Anderson S, Rennke HG, Brenner BM (1986) Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. J Clin Invest 77:1993–2000
18. Fabbri A, Cocchi R, Degli Esposti E, Lucatello A (1990) Antiproteinuric effect of angiotensin-converting enzyme inhibitors in patients with primary glomerular disease and normal renal function. Nephrol Dial Transplant [Suppl] 1:81–83
19. Remuzzi A, Puntoneri S, Battaglia C, Bertani T, Remuzzi G (1990) Angiotensin converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. J Clin Invest 85:541–549
20. Morelli E, Loon N, Myers BD (1989) Effect of converting enzyme inhibitor (CEI) on diabetic glomerular injury (DGI). Kidney Int 35:211
21. Remuzzi G, Ruggenenti P, Benigni A (1997) Understanding the nature of renal disease progression. Kidney Int 51:2–15
22. Iordache BE, Imberti O, Foglieni C, Remuzzi G, Bertani T, Remuzzi A (1994) Effects of angiotensin-converting enzyme inhibition on glomerular capillary wall ultrastructure in MWF/Ztm rats. J Am Soc Nephrol 5:1378–13
23. Meyer TW, Anderson S, Rennke HG, Brenner BM (1987) Reversing glomerular hypertension stabilizes established glomerular injury. Kidney Int 31:752–759
24. Heeg J, De Jong P, Vriesendorp R, De Zeeuw D (1990) Additive antiproteinuric effect of the NSAID indomethacin and the ACE inhibitor lisoprinosil. Am J Nephrol 10:94–97
25. Lapinski R, Perico N, Remuzzi A, Sangalli F, Benigni A, Remuzzi G (1996) Angiotensin II modulates glomerular capillary permselectivity in rat isolated perfused kidney. J Am Soc Nephrol 7:653–660
26. Mayer G, Lafayette R, Oliver J, William M, Myers B, Meyer T (1993) Effects of angiotensin II receptor blockade on remnant glomerular permselectivity Kidney Int 43:346–353