Effects of cyclosporin A on glomerular barrier function in the nephrotic syndrome

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1. To elucidate the mechanisms by which cyclosporin A diminishes proteinuria, we studied 20 patients with severe nephrotic syndrome. Biopsy-established pathologies included minimal change disease (n=5), membranous glomerulopathy (n=6), membranoproliferative glomerulonephritis (n=5) and focal segmental glomerulosclerosis (n=4). Before, at the end of a 90 day course of cyclosporin A, and finally 1 month after stopping cyclosporin A we determined 24 h protein excretion. Measurements of glomerular filtration rate, effective renal plasma flow, fractional clearance rates of albumin and immunoglobulins with different charges and the transglomerular sieving of uncharged dextrans of broad size distribution were used to study the effects of cyclosporin A on renal perfusion and the glomerular filtration barrier. The findings were analysed with a theoretical model of solute transport.

2. Among the different forms of glomerulopathy the response to low-dose cyclosporin A (trough levels 32.0–36.9 ng/ml) varied markedly. In minimal change disease, proteinuria decreased from 9.5±3.1 to 1.3±0.2 g/24 h (mean ± SEM, P<0.01). This response was due to restoration of the charge selectivity of the glomerular barrier. The depressed value of the glomerular permeability coefficient also returned to normal. Glomerular filtration rate, effective renal plasma flow and renal vascular resistance did not change. Proteinuria returned after stopping cyclosporin A, although it did not reach pretreatment levels. In membranous glomerulopathy, proteinuria fell from 9.9±1.5 to 1.8±0.3 g/24 h (P<0.01). Changes in protein excretion and dextran sieving were compatible with an increase in glomerular permeselectivity and a decrease in filtrate flow through the 'shunt' pathway. Glomerular filtration rate was maintained, although effective renal plasma flow fell significantly. Proteinuria relapsed after stopping cyclosporin A. In membranoproliferative glomerulonephritis and focal segmental glomerulosclerosis proteinuria did not respond to cyclosporin A, although cyclosporin A exerted important haemodynamic effects.

3. In minimal change disease and membranous glomerulopathy cyclosporin A exerts its beneficial effects on proteinuria through changes in the properties of the glomerular barrier, resulting in increased charge and size selectivity, respectively.

INTRODUCTION

In recent years a role has emerged for cyclosporin A (CsA) in the treatment of the nephrotic syndrome. Favourable effects of this drug have been reported in minimal change disease (MCD) [1], the Asian form of IgA nephritis [2] and membranous glomerulopathy (MG) [3]. As yet, the precise model of action of CsA in these disorders is the subject of intense debate. CsA is a potent suppressor of T-cell-derived lymphokines [4], which could play a role in the permeability of the glomerular basement membrane under pathological conditions [5]. On the other hand, CsA is known to exert distinct effects on renal perfusion and filtration through constriction of the afferent glomerular arteriole [4], which could, at least in part, contribute to the anti-proteinuric action of CsA by reducing net ultrafiltration pressure.

In an attempt to delineate the effects of CsA on ultrafiltration in more detail, we studied renal haemodynamics and glomerular basement membrane permeability in 20 patients with various forms of the nephrotic syndrome before and after a 12-week course of CsA therapy. The clearances of thalamate, hippurate and neutral dextrans of graded size, and the urinary excretion of albumin, IgG and IgG4, were used to study the effects of CsA on renal perfusion and the glomerular filtration barrier.

Part of this study was presented at the 11th International Congress of Nephrology, 15–20 July 1990, Tokyo, Japan.

METHODS

Patients and study design

Adult patients with the nephrotic syndrome and specific glomerular pathology that had been diagnosed by renal biopsy and who had no evidence of underlying systemic disease were eligible for the ongoing open trial,

Key words: glomerular sieving, membranous glomerulopathy, minimal change disease, permeselectivity, proteinuria.

Abbreviations: CI, charge index; CsA, cyclosporin A; ERPF, effective renal plasma flow; FSGS, focal segmental glomerulosclerosis; GFR, glomerular filtration rate; MCD, minimal change disease; MG, membranous glomerulopathy; MPGN, membranoproliferative glomerulonephritis; RVR, renal vascular resistance; SI, selectivity index.

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Table 1. Clinical characteristics of the patients

<table>
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<th>Serum creatinine concn. (μmol/l)</th>
<th>Serum albumin concn. (g/l)</th>
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in which the anti-proteinuric effects of CsA are being investigated. The criteria for inclusion were a urinary protein excretion rate of ≥ 3.5 g/day and a creatinine clearance of ≥ 40 ml/min. The use of immunosuppressive agents, angiotensin-converting enzyme inhibitors or non-steroidal anti-inflammatory drugs was not allowed in the 2 months before entry or during the 3 months of the study.

The present report includes 20 patients. A diagnosis of MCD was made in five patients (one patient was steroid-resistant and four were frequent relapsers), MG in six, membranoproliferative glomerulonephritis (MPGN) in five and focal segmental glomerulosclerosis (FSGS) in four. The clinical characteristics of the patients are shown in Table 1. All patients had evident peripheral oedema.

The study period consisted of a 2 month observation period followed by a 3 month CsA treatment period. CsA was taken at meal times in two separate doses. The use of immunosuppressive agents, angiotensin-converting enzyme inhibitors or non-steroidal anti-inflammatory drugs was not allowed in the 2 months before entry or during the 3 months of the study.

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The study period consisted of a 2 month observation period followed by a 3 month CsA treatment period. CsA was taken at meal times in two separate doses. The starting dose was 6 mg day⁻¹ kg⁻¹. Subsequent maintenance CsA therapy was adjusted to achieve trough levels of immunoassayable CsA in plasma of around 50 ng/ml. A specific monoclonal antibody (Cyclotrac; Incstar, Stillwater, MN, U.S.A.) was used to characterize the parent drug. Patients were monitored at 2-week intervals for 1 month and every 4 weeks until the third month or more frequently if indicated by their clinical status. Routine biochemistry, CsA trough levels and 24 h urinary protein excretion were assessed at these visits. Eight healthy subjects, who were normotensive, free of known renal disease and devoid of clinically measurable proteinuria, served as a control group for the differential dextran clearances.

### Procedures

Each patient was studied twice, at the start of the trial and after 3 months of treatment with CsA, after giving informed consent to the study procedures that had been approved previously by the Ethical Committee of University Hospital Dijkzigt. All patients were studied using the following clearance protocol.

After a light breakfast, patients drank tap water (20 ml/kg body weight) in 20 min. Plastic cannulas were then inserted into an antecubital vein of each arm. One arm was used for the infusion of dextrans and radiolabelled clearance markers, while blood samples were drawn from the other arm. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined with a constant infusion technique, by measuring the renal clearances of ¹²⁵I-iodothalamate and ¹³¹I-orthioiodohippurate (Amersham International, Amersham, Bucks, U.K.), respectively. The priming dose and sustaining infusion rate were adjusted for renal function using the following method. The GFR, and therefore the clearance of ¹²⁵I-iodothalamate, was estimated using the equation of Cockcroft & Gault [6]. The clearance of ¹³¹I-iodohippurate was estimated to be fivefold higher, assuming a filtration fraction of approximately 0.20. Infusion rates were then calculated to yield desirable steady-state levels. A suitable priming dose was calculated from the estimated steady-state levels and the volume of distribution. During the study period all patients remained in the supine position, but were allowed to stand in order to pass urine. After a 1 h equilibration period, the bladder was emptied by voiding. After a slow injection of 200 mg of dextran-10
Rheomacrodex; NPBI) were administered in a 10 min infusion. Urine was collected during three carefully timed 30 min intervals. Plasma and urine counts of $^{125}$I-iodothalamate and $^{131}$I-iodohippurate were measured in a $\gamma$-scintillation counter. The standard formula was used to calculate the clearance values. The mean GFR and EWF were calculated from the three clearance periods. Renal vascular resistance (RVR) was calculated as:

$$[(\text{MAP} - 10) \times (1 - \text{PCV})/\text{ERPF}] \times 22.16 \text{ (kdyn s cm}^{-2}\text{)}$$

where MAP is mean arterial pressure and PCV is packed cell volume. During the last collection period three plasma samples were drawn at the beginning, middle and end. These samples were combined in order to obtain an ‘average’ plasma concentration of dextran. Fractional clearances ($F_{CL,M}$) or in the case of dextran, sieving coefficients, of macromolecules [M] were calculated using the equation:

$$F_{CL,M} = \left\{ \frac{[U/P]_M}{[U/P]_M^{125\text{I}-\text{iodothalamate}}} \right\}$$

in which $U$ and $P$ denote the urinary and plasma concentrations of the macromolecule and $^{125}$I-iodothalamate. In the urine and plasma samples used for the measurement of dextrans we also measured albumin, IgG and IgG$_s$ concentrations. The selectivity index (SI) was calculated as IgG clearance/albumin clearance. In general, proteinuria is considered to be selective when the SI is below 0.2. As IgG and IgG$_s$ are differently charged at physiological pH, IgG clearance/IgG$_s$ clearance can be used as an indicator of charge selectivity (the charge index, CI) [7].

**Laboratory methods**

Blood pressure was determined with an oscillometric device (Accutorr; Datasonics Corp, Paramus, NJ, U.S.A.). The means of five consecutive readings after a 15 min ‘run-in’ period were used for analysis.

Serum creatinine concentrations were measured using a modified Jaffé method. Total urinary protein concentrations and albumin concentrations in plasma and urine were measured using an immunoturbidimetric assay. For the determination of IgG and IgG$_s$ in plasma and urine, a sandwich radioimmunoassay was developed. In brief, Maxisorb test tubes (Nunc, Roskilde, Denmark) were coated with a monoclonal anti-IgG or anti-IgG$_s$ (MH16-01M and MH164-1, respectively; Central Laboratory for Blood Transfusion, Amsterdam, The Netherlands). After overnight incubation with sufficiently diluted samples, the tubes were washed carefully. Another monoclonal anti-IgG or IgG$_s$ (MH16-02M or MH164-4; Central Laboratory for Blood Transfusion) radiolabelled with $^{125}$I was added and after washing, the tubes were assayed for IgG or IgG$_s$ using reference serum (0-001; Central Laboratory for Blood Transfusion) as the standard. The sensitivity of both assays was 1 ng/ml. Interassay coefficients of variation were 6.4 and 7.5%, respectively. Dextran was assayed after protein-free filtrates of plasma and urine had been separated into narrow fractions by gel-permeation chromatography using the method described by Granath & Kvist [8]. A Sephacryl S-300 column (Pharmacia, Uppsala, Sweden) was used with a bed volume of 180 ml and a length of 90 cm. The eluent was 0.01 mol/l Tris buffer with 0.15 mol/l NaCl and 1 mmol/l EDTA at pH 7.0. Blue Dextran was used to determine the void volume ($V_0$) and the column was calibrated using dextran-10, -40 and -70. The fractional volume available to the solute ($K_{av}$) was then calculated from:

$$K_{av} = (V_c - V_0)/(V_t - V_0)$$

where $V_c$ is the elution volume of the solute and $V_t$ is the total bed volume of the gel column. Effective molecular radii for the individual dextran fractions were calculated from $K_{av}$. After gel-permeation chromatography, eluted fractions were assayed for dextran by the anthrone method of Scott & Melvin [9]. Afferent colloid osmotic pressure ($\pi_a$) was calculated using the formula of Landis & Pappenheimer [10].

**Analysis of glomerular membrane pore structure**

To analyse the size-selective properties of the glomerular barrier, we used a heteroporous model of the glomerular capillary wall, as described by Deen et al. [11]. This model has been shown to provide the most satisfactory representation of dextran sieving. In this model the major portion of the capillary wall is perforated by restrictive cylindrical pores of identical radius ($r_o$). In addition, this model assumes a parallel ‘shunt pathway’ that does not discriminate on the basis of dextran size and through which a small fraction of the filtrate volume ($\omega$) passes. This fraction is not merely dependent on changes in the properties of the capillary wall, but also on intra-capillary oncotic pressure. Therefore a quantity closely related to $\omega$, but characteristic of the membrane $per se$, is used. This quantity ($\omega_0$) is the fraction of the volume flux that would pass through the shunts if plasma proteins were absent. The membrane barrier to dextrans is fully characterized by $r_o$, $\omega_0$ and $K_t$, where $K_t$ is the product of effective hydraulic permeability and glomerular capillary surface area. An important supposition in these calculations is the value of the trans-membrane hydraulic pressure difference ($\Delta P$). Since $\Delta P$ cannot be measured directly in humans, a value must be assumed in order to calculate the basement membrane parameters. In normal subjects $\Delta P$ is predicted to be close to 35 mmHg [12], whereas in most forms of renal disease $\Delta P$ appears to be elevated to approximately 40 mmHg. We therefore calculated intrinsic basement membrane parameters using a $\Delta P$ of 35 mmHg in healthy control subjects and 40 mmHg in the patients with various forms of glomerulopathy. The unavailability of measurements of $\Delta P$ in humans does not seriously hamper the use of dextran.
Thus, although the quantitative changes in basement membrane characteristics were small, in each group the difference between baseline and CsA periods was tested parametrically using the Wilcoxon rank-sum test. As the numbers in the separate groups were small, each group the difference between baseline and CsA periods was tested parametrically using the Wilcoxon rank-sum test. Results are expressed as means ± SEM.

Statistical analyses

Differences between the four diagnostic groups were analysed using analysis of variance and, when this produced a significant F value, it was followed by the Student–Newman–Keuls test. The Student–Newman–Keuls test. As the numbers in the separate groups were small, each group the difference between baseline and CsA periods was tested parametrically using the Wilcoxon rank-sum test. Results are expressed as means ± SEM.

RESULTS

At the low doses used, side-effects of CsA were minimal and consisted of a slight rise in blood pressure in some patients. No anti-hypertensive treatment was required. All patients completed the 12 week course of therapy according to the protocol. The mean plasma levels of CsA were comparable in all groups and averaged 34.2 ng/ml. Before treatment proteinuria was massive with no significant difference between the various diagnostic groups. The anti-proteinuric effect of CsA, however, varied markedly.

MCD

As expected, proteinuria in these patients was highly selective. Urinary excretion rates of albumin and IgG were 4323 ± 550 and 64 ± 24 µg/min, respectively, and SI was 0.158 ± 0.080 (Table 2). Renal function was not depressed, as GFR and ERPF averaged 100 ± 10 and 631 ± 54 ml/min, respectively (Table 3) and the mean serum creatinine concentration was 70.6 ± 4.6 µmol/l. Blood pressure was normal in these patients. The normal size selectivity of the proteinuria was also reflected by the baseline dextran sieving pattern, as the FcL of large dextrans was comparable with that of normal subjects. However, the FcL of smaller dextrans (2.8–3.2 nm) was lower than that in healthy control subjects, which is compatible with a 53% decrease in FcL (Table 4).

After treatment with CsA, proteinuria was markedly decreased (Fig. 1) and the FcL values of albumin and IgG were lowered by 92 and 87%, respectively, and SI was 0.158 ± 0.080 (Table 2). Renal function was not depressed, as GFR and ERPF averaged 100 ± 10 and 631 ± 54 ml/min, respectively (Table 3) and the mean serum creatinine concentration was 70.6 ± 4.6 µmol/l. Blood pressure was normal in these patients. The normal size selectivity of the proteinuria was also reflected by the baseline dextran sieving pattern, as the FcL of large (5.0–5.8 nm) dextrans was comparable with that of normal subjects. However, the FcL of smaller dextrans (2.8–3.2 nm) was lower than that in healthy control subjects, which is compatible with a 53% decrease in FcL (Table 4).

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explain the altered dextran sieving without changing $K_r$. No significant changes occurred in $r_0$ or $\omega_0$. Treatment with CsA was discontinued in four out of five patients, after which proteinuria increased towards, although not reaching, baseline values.

**MG**

The amount of proteinuria was comparable with that in MCD. However, proteinuria was non-selective, as the urinary excretion rate of IgG was $207 \pm 49 \, \mu g/min$ and SI was $0.290 \pm 0.052$ (Table 2). GFR in MG was lower than in MCD ($87 \pm 14$ versus $100 \pm 10 \, ml/min$, $P<0.05$) and the serum creatinine concentration averaged $96 \pm 6.1 \, \mu mol/l$. ERPF, filtration fraction and blood pressure did not differ significantly from MCD. The decrease in size selectivity was also reflected by dextran sieving. The $F_{CL}$ of dextrans with a small radius were lower and the $F_{CL}$ of those with large radii were higher when compared with normal subjects. $K_r$ was lower and shunt flow was higher than in control subjects (Table 4). In MG, CsA induced a decrease in protein excretion from $9.9 \pm 1.2$ to $1.8 \pm 0.3$ g/24 h ($P<0.01$). The $F_{CL}$ of albumin and IgG decreased by $68$ and $84\%$ ($P<0.05$), respectively. Serum albumin concentration rose from $25.3 \pm 2.0$ to $37.0 \pm 1.2$ g/l ($P<0.01$). After CsA, SI decreased by $30\%$ ($P<0.05$), whereas charge selectivity remained unaltered. GFR was not significantly decreased, but ERPF was decreased by $35\%$ ($P<0.05$). Filtration fraction rose by $21\%$ ($P<0.05$). Mean blood pressure remained unchanged but RVR was elevated by $55\%$ ($P<0.05$). CsA significantly increased the $F_{CL}$ of dextrans with radii of 2.8 and 3.0 nm and decreased the $F_{CL}$ of those with a radius of 5.2-5.8 nm (Fig. 2). These findings fit well in the observed decrease in SI. The calculated basement membrane parameters indicate that glomerular permselectivity increased as $\omega_0$ was decreased by CsA, from $8.0 \pm 1.7 \times 10^{-3}$ to $1.6 \pm 0.6 \times 10^{-3}$ ($P<0.05$). The assumption of $\Delta P$ did not markedly influence the calculated value of $\omega_0$. $K_r$ was not significantly altered by CsA in MG. Proteinuria increased towards baseline levels in the four patients in which treatment was discontinued (Fig. 1).

**MPGN**

In these patients proteinuria was equal to that in patients with MCD and MG. Renal function was markedly depressed, as GFR was $53 \pm 13 \, ml/min$, a value lower than in MCD and MG ($P<0.05$). Blood pressure was higher than in these two groups ($P<0.05$). Proteinuria was non-selective (SI $0.441 \pm 0.141$) and baseline dextran sieving was also compatible with decreased size selectivity.

After treatment with CsA, GFR and ERPF were decreased by 15 and 28%, respectively, and filtration fraction remained unchanged. Mean blood pressure increased by 11% and RVR increased by 62%. In spite of these effects on haemodynamics, no changes were observed in proteinuria and albumin or IgG excretion (Fig. 1, Table 2). The serum albumin concentration was not altered by CsA ($22.0 \pm 1.9 \, g/l$ before and $23.4 \pm 2.6 \, g/l$ after treatment). Also, no changes in dextran sieving or glomerular basement membrane permeability characteristics were observed (Fig. 2).

**FSGS**

In the baseline situation, proteinuria was comparable with that in the other groups. GFR was lower than in patients with MCD ($70 \pm 2 \, ml/min$, $P<0.05$), but did not differ significantly from the other patient groups. Proteinuria was non-selective, as indicated by both a SI of $0.292 \pm 0.012$ and a dextran sieving pattern comparable with that in MG and MPGN. Patients with FSGS
Control subjects
MG
FSGS
MPGN
responded to CsA in a fashion similar to those with MPGN with significant changes in GFR, ERPF and RVR which were not accompanied by changes in protein excretion or serum albumin concentration (Tables 2 and 3). CsA did not alter dextran sieving in FSGS.

**DISCUSSION**

Since the original report by Meyrier et al. [13] in 1986 that CsA could diminish proteinuria in some nephrotic patients, the number of studies on this subject has steadily increased [1–3, 14, 15]. In particular, in corticosteroid-sensitive and multi-relapsing cases, CsA appeared to be effective [1]. However, the results of many of these pilot studies are open to criticism, as they differed widely in terms of objectives, population studied, CsA dosage, effects on renal function and concomitant immunosuppressive treatment. Our previous findings of a pronounced effect of CsA on proteinuria in patients with Alport’s syndrome, a non-immunological glomerular disease, suggested that CsA could operate, at least in part, without interfering with a specific immunological mechanism [16]. From experience with CsA in organ transplantation and autoimmune disorders such as uveitis [17] and psoriasis [18], it appears that CsA can induce intense, dose-dependent renal vasoconstriction, probably at the pre-glomerular afferent arteriole. Theoretically, predominant afferent arteriolo-constriction can reduce the net ultrafiltration pressure and thereby proteinuria. Indeed, the findings of Myers et al. [19], who observed a trend towards restricted trans-glomerular transport of neutral dextrans of graded size in CsA-treated heart transplant recipients, and measurements by Barros et al. [20] of glomerular haemodynamics, in Munich–Wistar rats, were evidence in favour of an effect of CsA on $K_f$ and/or trans-membrane hydraulic pressure. Thus, of the four determinants of glomerular ultrafiltration, i.e. (1) the rate of nephron plasma flow, (2) the afferent oncotic pressure, (3) the glomerular hydrostatic pressure gradient and (4) the glomerular permeability coefficient, CsA could have an effect on at least three. In the present study we deliberately opted for a relatively low-dose regimen in order to minimize renal side-effects. Doing so we found that the anti-proteinuric effects of CsA (plasma trough levels around 35 ng/ml) varied markedly among the different forms of glomerulopathy.

In our patients with MCD the anti-proteinuric effects of low-dose CsA were most striking, as complete remission was obtained in three out of five patients. Before CsA, in the baseline untreated state, GFR, ERPF and blood pressure were normal and the massive proteinuria was highly selective. The $F_{CL}$ of large dextrans with radii > 4.0 nm was comparable with that in normal subjects. In contrast, the sieving of small dextrans was hindered. By applying our dextran-sieving data to a well-described and applied theoretical model of solute transport, we could explain the abnormal sieving of small dextrans in this condition by a reduction in $K_f$, the product of hydraulic permeability and capillary surface area. Similar findings have been reported by Bridges et al. [21].

The marked reduction in protein excretion observed in MCD after CsA was due to the restoration of the charge selectivity of the glomerular basement membrane. Serum creatinine concentration, GFR and blood pressure did not change, although ERPF and RVR showed non-significant downward and upward trends, respectively. Filtration fraction rose significantly. Likewise, the hampered sieving of small dextrans was improved by CsA, although values did not reach the level observed in normal control subjects. Calculation of membrane parameters indicated that the raised $F_{CL}$ of small dextrans can be accounted for by a rise in $K_f$. A raised $K_f$ implies the emergence of more pores or an augmented glomerular capillary surface area. Such effects are difficult to reconcile with a vasocostric-tory effect of CsA. Unfortunately, a pronounced decrease in $\Delta P$ is predicted to have similar effects on dextran sieving to those of a rise in $K_f$ [22]. However, if $K_f$ is assumed to remain unchanged after CsA, a decrease in $\Delta P$ to 28 mmHg would be required to explain our results. In a long-term study performed in heart transplant recipients, the changes in dextran sieving were indeed compatible with a decrease in $\Delta P$ to 30 mmHg [23]. However, in these patients ERPF and GFR were halved. In our patients the haemodynamic changes were less

| Table 4. Membrane parameters in control subjects and in patients with various forms of the nephrotic syndrome at baseline and after 3 months of CsA. Values are means (SEM). Statistical significance: *P < 0.05 versus control subjects; †P < 0.05 versus baseline. Calculations were made using a $\Delta P$ of 35 mmHg in healthy control subjects and 40 mmHg in various disease states. |
|-----------------|-----------------|-----------------|
|                 | $K_f$ (ml min$^{-1}$ mmHg) | $r_0$ (mm) | $10^8 \times \sigma_p$ |
| Control subjects| 13.3 (0.9)       | 5.69 (0.01)    | 1.4 (0.1) |
| MCD             | Baseline 6.3* (1.9) | 5.57 (0.01)    | 2.5 (0.4) |
|                 | CsA 9.8† (1.3)   | 5.53 (0.01)    | 1.4 (0.2) |
| MG              | Baseline 4.8* (1.6) | 5.55 (0.02)    | 8.0* (1.7) |
|                 | CsA 6.7 (2.0)   | 5.62 (0.03)    | 1.6† (0.6) |
| MPGN            | Baseline 3.5* (1.4) | 5.58 (0.02)    | 11.3* (3.2) |
|                 | CsA 2.6 (1.2)   | 5.57 (0.01)    | 10.6 (3.0) |
| FSGS            | Baseline 3.9* (2.6) | 5.49 (0.02)    | 8.9* (1.6) |
|                 | CsA 3.0 (2.4)   | 5.62 (0.03)    | 9.2 (2.6) |
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Fig. 1. Proteinuria at baseline, during 3 months of treatment with CsA and 1 month after discontinuing treatment in patients with various forms of nephropathy. (a, MCD; b, MG; c, FSGS; d, MPGN) CsA was not discontinued in one patient with MCD and two patients with MG.

impressive and filtration fraction actually increased. Therefore such a fall in \(\Delta P\) seems very unlikely in our patients. It is noteworthy that comparable changes in the sieving of small dextrans in MCD were described after treatment with prednisone [24], a drug without an important haemodynamic mode of action. Therefore treatment with CsA seems to result in remission of the disease, with normalization of the basement membrane characteristics.

Current understanding of the pathophysiology of MCD focuses on a circulating 'permeability' factor that somehow alters the negative charge of the glomerular basement membrane [25]. It has been suggested that this factor
could be a T-cell-derived lymphokine [5, 26]. As CsA inhibits the production of lymphokines by T-cells [4], our findings of a recovery of charge selectivity during CsA and the prompt recurrence of proteinuria after stopping CsA are compatible with this hypothesis.

CsA also had a pronounced effect on proteinuria in patients with MG. However, its model of action on the diseased glomerular barrier was different from that in MCD. Before CsA, in the untreated state, proteinuria was non-selective and the clearance of $^{125}$I-iodothalamate, the $F_{Ct}$ of uncharged dextran with a radius between 2.8 and 3.8 nm and filtration fraction were depressed significantly below values in our healthy subjects. In contrast, the passage of dextran with radius $>5.4$ nm was increased. Applying the theoretical model of glomerular solute transport of Deen et al. [11] to these findings, the abnormal dextran sieving can be explained by a loss of intrinsic ultrafiltration capacity (depressed $K_t$) and

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Fig. 2. $F_{Ct}$ of neutral dextran measured before ($\circ$) and after 3 months treatment with CsA ($\star$) in patients with various forms of nephropathy. (a, MCD; b, MG; c, FSGS, d, MPGN). Values are means ± sem. Statistically significant: $^*P < 0.05$ versus baseline.
decreased barrier size selectivity. Using a $\Delta P$ of 40 mmHg, we calculated $K_f$ to be depressed threefold and the fraction of filtrate permeating the shunt pathway ($\omega_0$) to be increased fivefold.

In contrast with the effects of CsA on charge selectivity in patients with MCD, the anti-proteinuric action of CsA in MG was the result of an increase in size selectivity of the glomerular filtration barrier. Blood pressure and GFR did not change significantly, although ERPF fell markedly by 35%. The $F_C$, of dextrans measuring 2.8-3.2 nm rose and that of large dextrans (5.0-5.8 nm) fell. Our computations revealed that the changes in glomerular sieving induced by CsA could be accounted for by a rise in $K_f$ and a pronounced diminution of filtrate passing through the 'shunt' pathway, $\omega_0$. Little or no effect on $\omega_0$ was predicted to occur when we varied $\Delta P$ throughout a wide range, between 24 and 52 mmHg. Thus, the unavailability of data on $\Delta P$ in humans does not seriously influence our interpretations.

Theoretically, a CsA-induced decrease in $\Delta P$ could also alter the structure of the glomerular basement membrane and thereby its permeability characteristics [27]. Indeed, significant changes in permselectivity were observed in patients with glomerulopathy during treatment with drugs that decrease filtration pressure, such as indomethacin [11] and angiotensin-converting enzyme inhibitors [28]. These changes, however, were unvaryingly accompanied by a decrease in filtration fraction. In our CsA-treated patients filtration fraction rose, suggesting that glomerular haemodynamics per se cannot explain the anti-proteinuric action of CsA. We therefore conclude that non-haemodynamic factors, most likely of immunological origin, could be involved in the beneficial effects of CsA on proteinuria in MG.

In FSGS and MPGN, proteinuria was non-selective from the onset and remained so after treatment. No effect of CsA on proteinuria was observed in these patients. After CsA, renal haemodynamics were severely disturbed in patients with FSGS and MPGN, with both GFR and ERPF dropping markedly and RVR rising. Interestingly, blood pressure also increased significantly in patients with FSGS and MPGN whereas no change in blood pressure was observed in patients with MCD and MG. This is in accordance with the previous report that patients who do not respond to treatment with CsA are more likely to be affected by its side-effects [1]. It also argues against a haemodynamic mode for the anti-proteinuric action in the responding patients, as the patients most severely affected by haemodynamic changes had no change in protein excretion.

If CsA is to be of clinical importance in the nephrotic syndrome, it is essential that its effects are not solely haemodynamic in nature. If the symptomatic reduction of proteinuria were the objective, the current therapeutic arsenal hold several drugs, such as indomethacin [29] and angiotensin-converting enzyme inhibitors [30], that probably decrease proteinuria through haemodynamic mechanisms but are less nephrotoxic with continued treatment. The results of the present study demonstrate that, in MCD and MG, low doses of CsA exert a dramatic effect on proteinuria with relatively minor effects on renal haemodynamics. Furthermore, the decrease in renal function did not appear to be instrumental in reducing proteinuria. Therefore, in MG and MCD, the immunosuppressive action of CsA may play an important role in decreasing proteinuria.

In all patients in which CsA was discontinued, proteinuria returned promptly. At present it is unclear whether a lasting remission can be obtained with longer treatment and several authors have suggested that prolonged treatment with low doses of CsA (2-4 mg day$^{-1}$ kg$^{-1}$) is required to maintain remission [1,3]. However, it remains to be demonstrated that these lower doses are free of long-term nephrotoxic side-effects, as evident morphological changes were observed at doses as low as 5 mg day$^{-1}$ kg$^{-1}$ in heart transplant recipients [23]. Therefore caution is in order for the long-term use of CsA in diseases with a relatively benign course, such as MCD and MG.

We conclude that in MG CsA exerts its effects on proteinuria mainly through a change in the permeability characteristics of the glomerular basement membrane resulting in a decrease in shunt-flow, despite increased filtration fraction. In MCD, CsA decreases proteinuria through an increased charge selectivity and appears to increase $K_f$.

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