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PROTEINS, GROWTH FACTORS, AND PROGRESSION OF KIDNEY DISEASE

PROTEINURIA IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS: ROLE OF CIRCULATING FACTORS AND THERAPEUTIC APPROACH

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ABSTRACT

The clinical course of primary Focal Segmental Glomerulosclerosis (FSGS) is frequently complicated by nephrotic range proteinuria and progression to renal failure. The high recurrence rate of the disease in transplanted kidney suggests the hypothesis that such patients have a circulating factor that alters glomerular capillary permeability. In recent years some authors found that serum from patients with FSGS increases glomerular permeability to albumin and partially identified the permeability factor (PF) as a protein of 30–50 Kd m.w. The removal of this protein by means of Plasma Exchange (PE) or plasma Immunoadsorption by Protein A (IA) decreased proteinuria. In this report we provide preliminary data about the prevalence of PF and the therapeutic effect of its

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removal by IA, in 3 pts with recurrence in the transplanted kidney, and 4 with FSGS of the native kidneys. They were resistant to corticosteroids (CS) and immunosuppressive (IS) therapy. 10 IA sessions were performed in 4 weeks: if a remission was achieved IA was gradually tapered. The level of PF in the serum was measured by an in vitro assay to determine the glomerular permeability to albumin. The FSGS was histologically proven in all cases and the degree of evolution was evaluated. PF levels, serum creatinine, daily proteinuria and serum albumin were monitored. The 3 patients with recurrent FSGS had a normalization of the PF levels; 2 had a clinical remission. In FSGS of native kidneys PF was elevated in 3/4 cases; 1 had a clinical remission; 2 with extensive sclerohyalinosis and 1 without PF levels did not improve. Our results confirm that most patients with FSGS have high PF serum levels and suggest that its removal can be beneficial.

Key Words: FSGS; Proteinuria; Permeability factor; Protein A immunoabsorption

INTRODUCTION

Approximately 76% of children and 55% of adults affected by primary Focal Segmental Glomerulosclerosis (FSGS) present nephrotic syndrome (1). 50% of patients with proteinuria in nephrotic range (> 3,5 g/24 h) will develop terminal renal failure after 6–8 years (2). FSGS re-appears in 15–50% of transplanted kidneys and a half of these cases have renal function loss (3,4). Studies carried out since the Seventies revealed the presence in FSGS patients of blood factors (5), called ''proteinuric'' or ''permeability'' factors (PF), which have partially been isolated only in the last 10 years (6–8). Although the exact chemical-physical features and biological behavior of these factors are still unknown, they are assumed to be proteins, because of the abolition of their activity after boiling, and their precipitation in ammonium sulfate. Recently published data seem to indicate that the presence of circulating PF has a significant predictive value for the recurrence of proteinuria in children with FSGS who have received a renal allograft (9). The possibility of circulating PF has fostered the use in these patients of therapeutic apheresis techniques: published studies have demonstrated that conventional Plasma Exchange (PE) and Protein A immunoadsorption (lA) reduce the permeability activity in patients' plasma and decrease proteinuria (9–14).

METHODS

Measurement of Albumin Permeability Factors: the presence of PF was evaluated by an vitro model (7): by placing decapsulated glomeruli in a 5%

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bovine serum albumin medium, no variation in glomerular volume appears, but if we put the glomeruli in a 1% albumin medium, an oncotic gradient will appear. If the glomerular capillary wall is normally resistant to albumin, the water will flow inside the capillary and the glomerular volume will increase, but in the presence of altered capillary permeability induced by patient sera, proteins will escape from the intracapillary glomerular tuft and the glomerular enlargement will be reduced. The measurement of variations in glomerular volume are described by Michele Carraro et al (9): the convective permeability to albumin (P alb) is defined by the formula: P alb $= 1 - \sigma$ alb, where σ represents the reflection coefficient to albumin, and include values from 0 to 1: P alb values of 0.6 or greater are considered positive for the presence of PF.

Kidney biopsies: the diagnosis was histologically proven, by means of current optical and immunofluorescence microscopy in all cases; electron microscopy in 3 cases. The prevalence of the sclerohyalinotic lesions was evaluated, to distinguish between early and advanced forms of FSGS.

Protein A Immunoadsorption (IA): the separated plasma was passed through two protein A containing columns (Immuno-adsorba, Excorirn, Lund, Sweden), which are alternatively perfused by plasma and regenerated, so that the protein A binding capacity is indefinitely prolonged and allows to treat 2 or more plasma volumes on each apheresis session. In all cases 10 IA sessions were performed in the intensive period of treatment (4 weeks): if no remission was achieved, the treatment was suspended; if a remission was achieved, the IA sessions frequency was gradually reduced.

PATIENTS

Seven patients (5 men and 2 women), 3 affected by FSGS in transplanted kidney, (1st group, cases l, 2, 3) and 4 by FSGS in native kidneys (2nd group, cases 4, 5, 6, 7), were studied from October 1995 to April 1999. Patients from the 1st group, 3 men, age from 22 to 31 yrs, started the dialytic treatment for histologically proven FSGS. All of them had recurrence of the disease within 1 year after cadaveric kidney transplantation. The P alb values indicated the presence of circulating PF. The kidney biopsy revealed in case 1 advanced FSGS and also chronic rejection grade II-III of the Banff classification; in case 2 partially evoluted FSGS and in case 3 early form of FSGS and a slight degree of CsA toxicity. Immunosuppression after transplantation consisted of standard double therapy with CS and Cyclosporin (CsA) in case 1 and 3 and triple therapy with CS, CsA, Azathioprine (AZA) in case 2. Despite intensification of immunosuppressive regimen, the patients showed worsening of proteinuria and renal function, and IA was associated to the drug therapy.

Patients from the 2nd group were 2 men and 2 women, age ranging from 18 to 60 years. Case 4 and 5 developed nephrotic syndrome (NS) with normal

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renal function; the kidney biopsy revealed early FSGS. Case 6 presented with NS and renal failure; renal biopsy showed FSGS in late stage. Case 7 presented with NS and mild renal insufficiency; the renal biopsy revealed partially advanced FSGS. The PF test was positive in cases 4, 6, 7. All patients have been treated with CS, (i.v: pulses and/or high oral daily dosage); case 4 received CsA for 4 months; case 5 underwent a brief cycle with AZA, discontinued for leukopenia; case 6 was treated with CsA for a short period because of renal failure; in case 7 Cy was added to CS therapy, but suspended after 1 month due to leukopenia. All cases did not respond to drug therapy. When starting IA sessions, the immunosuppressive therapy was reduced or gradually discontinued.

RESULTS

In this study, we took into consideration the variations in proteinuria, creatininemia, albuminemia, PF levels in serum and in the protein A column eluates, during a single IA session, at the end of the intensive period, and at the end of the general follow up.

The findings relating to the first two weeks of treatment (6 IA sessions) in patient 1, whose renal biopsy revealed a late stage FSGS and chronic rejection are showed in Figure 1. In this patient, despite normalization of serum PF levels (normal value $<0{,}6$), renal function and proteinuria do not go through any variation.

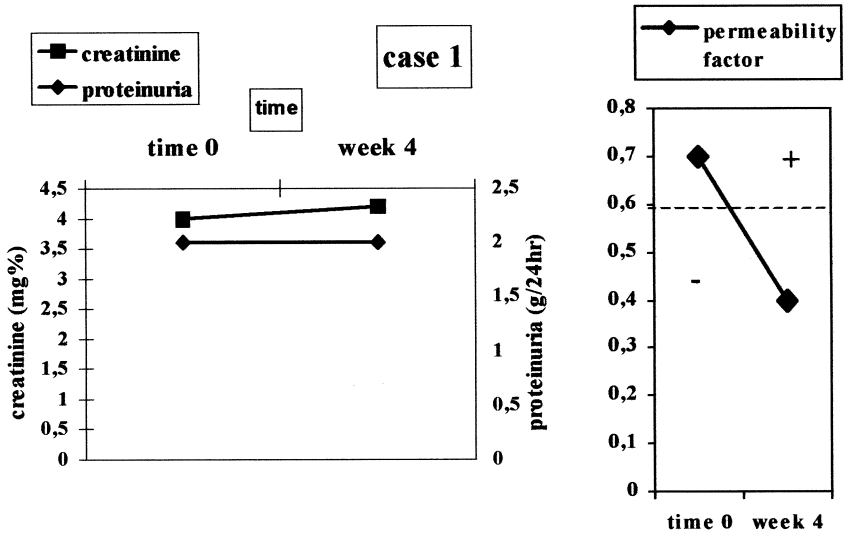

Figure 1. Patient 1 (relapse of FSGS in renal transplant and chronic rejection) after the first two weeks of IA treatment: renal function and proteinuria do not improve despite normalization of serum PF levels (normal value $<0{,}6$).

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In patients 2 and 3 proteinuria and albuminemia reach a non-nephrotic and normal level respectively, and renal function improves, the creatinine dropping from 1.8 to 1.6 mg/dL in patient 2 and from 3.8 to 1.5 mg/dL in patient 3. Figure 2 shows the results of a single IA session in the 2 patients with regard to the permeability test: serum PF is present at the beginning of the session, disappears at the end of it and, in both cases, is present in column eluate.

In regard to general follow-up, we obtained the following results:

- in patient 1, the IA treatment has been suspended after the intensive period due to the absence of a therapeutic response; the patient went back to dialysis after three months;
- patient 2 (follow-up: 40 months) continued the apheretic treatment with one session fortnightly: proteinuria and albuminemia levels maintained the same values as at the end of the intensive period, and creatininemia appeared slightly increased, i.e. 2.2 mg/dL. A 3-to-4 week interval between each IA session would cause an increase in proteinuria;
- patient 3 (follow-up: 11 months) undergoes an IA session every 2 to 3 weeks showing the same results as at the end of the intensive period. Following recurrent check ups run at the beginning of each IA session, patients 2 and 3 still show positive PF.

Patients 4, 5, 6, and 7 presented FSGS in native kidneys. The first 3 patients didn't show remission at the end of the intensive period and IA treatment was suspended in all of them. At the end of the follow-up, patients

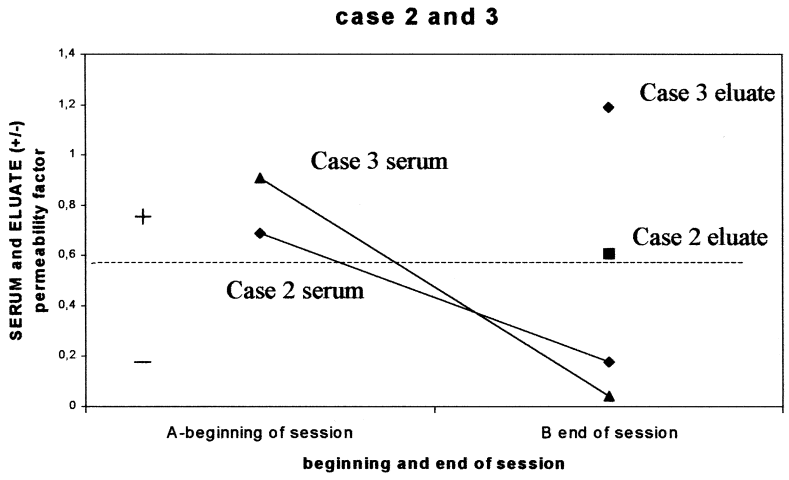

Figure 2. Behavior of the permeability factor (PF) before and after a single IA session in 2 patients: serum PF is present at the beginning of the session, disappears at the end of it and, in both cases, is present in column eluate.

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4 and 5 (who had negative PF test) still show proteinuria in nephrotic range and a slight increase in creatininemia; patients 6, the most severe case at biopsy, showed a faster worsening of renal function leading, after six months, to regular dialysis treatment.

Patient 7, in the acute stage, had a good response to IA treatment, showing normalization of PF and creatininemia levels and a clear decrease in proteinuria. These results persist after two months and after a 25-month follow-up period and, although increased at the end of this period, creatininemia still stays lower than starting values (Figure 3).

In this case, after the intensive period, IA sessions went on weekly for two months and fortnightly for one month before being suspended. A starting high CS dosage has been maintained for two months to be then gradually reduced to a final low dosage.

DISCUSSION

The above mentioned findings regard two different groups, the first including the 3 patients who presented relapsing FSGS in transplanted kidney, and the second including four patients affected by FSGS in native kidneys. In regard to PF, it was positive in all the first 3 patients, appearing in protein A column eluate and normalizing in serum. This PF behavior, in patients 2 and 3, was correlated to a decrease in proteinuria, normalization of serum albumin levels, and an improvement of renal function. Such a result well matches the description of literature case histories (6,7,9) and strengthens the idea according to which a circulating factor, able to make

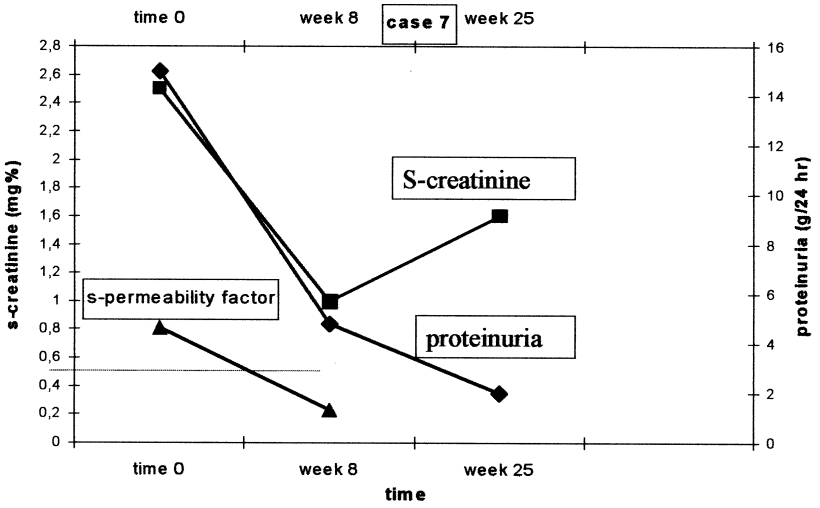

Figure 3. Patient 7 (partially advanced FSGS in native kidney): PF, renal function and proteinuria during IA treatment and clinical follow-up show a satisfactory result.

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in vitro glomerular capillaries permeable, is responsible for proteinuria in FSGS (6–9). Patient 1 didn't have a favorable clinical course: renal biopsy revealed a late stage FSGS, chronic rejection, and advanced renal failure, hence prognosis could not be other than poor. Patient 2 and 3, instead, achieved a partial remission, which is to be considered a positive result, most of all in patient 3 whose CsA toxicity made necessary a major reduction of CsA dosage in treatment. In both patients the satisfactory result achieved with IA treatment can only be maintained through long-term treatment at reduced frequency which, at the moment, still represents an unsolved problem.

With regard to the second group, i.e. the 4 patients affected by FSGS in native kidneys, results differ completely from those achieved in group 1. Indeed, only patient 7, treated with IA, achieved a partial remission lasting after suspension of apheretic sessions. In patients 4, 5, and 6, the IA intensive period didn't modify either proteinuria or renal function and patient 6 had rapidly to undergo dialytic treatment. The different patient clinical courses are due to differences existing amongst patients. These differences relate to:

Nephropathy stage: in the 2 cases evolving into dialytic treatment, i.e. in patients 1 and 6, renal biopsy had revealed a FSGS form evolving towards sclerohylinosis. Patient 1 also presented chronic rejection;

PF presence in serum: although affected by an early stage FSGS, patient 5 didn't respond to treatment and was the only one whose serum PF test was negative;

Drug therapy associated to IA sessions: the 3 patients affected by relapsing FSGS in transplanted kidney were all treated with immunosuppressors and high-dosage CS; the remaining 4 patients received lower doses of immunosuppressors and CS and, in any case, their therapy was being reduced at the beginning of the IA treatment cycle. Patient 7 only – partially advanced FSGS, positive PF, high-dosage CS during IA cycle – achieved a satisfactory result.

According to the above mentioned findings, and despite the limitations of this study, it is possible to hilight some useful data in determining the effectiveness and the use of IA in FSGS in native kidneys and in relapsing FSGS in the transplanted kidney that is unresponsive to treatment with CS, Cy, CsA, and AZA:

1. Renal biopsy is fundamental: only early or partially advanced forms of FSGS are potentially responsive. In the transplanted kidney, the presence of Banff grade II or III chronic rejection, probably makes IA useless;
2. FSGS forms that are negative to permeability testing are likely to be non-responsive to IA apheretic treatment;

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3. Immunosuppressive and CS treatment should be administered at high dosages to patients with recurrent FSGS in the transplanted kidney. Similarly patients with FSGS in their native kidney should be treated with immunosuppressants and CS at proper dosages, and this should be continued during the IA cycle.

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