Plasma immunadsorption treatment in patients with primary focal and segmental glomerulosclerosis

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Abstract

Background. In primary focal and segmental glomerulosclerosis (FSGS) renal prognosis is poor if no remission of proteinuria can be obtained by treatment. In some patients a permeability factor, responsible for damaging the glomerular epithelial cell and detectable by an in vitro test (GVV-test), seems to be present in the serum.

Method. We determined the effects of an immunadsorption treatment (IAT) on proteinuria and glomerular permselectivity (using a neutral dextran and dextran sulfate-sieving technique to assess glomerular size and charge selectivity) in five patients with FSGS in the native kidneys and three patients with recurrence of FSGS after kidney transplantation. Furthermore, we performed the GVV-test using sera obtained from the patients before and after therapy.

Results. IAT reduced proteinuria by more than 50% in four patients, all of whom had an improvement in glomerular-size selectivity. Charge selectivity was better preserved after therapy in three out of these four subjects. The GVV-test prior to IAT was positive in two patients who also responded clinically to therapy. After IAT the GVV-test was negative in all patients, indicating an elimination of the proteinuric factor in the two previously positive patients.

Conclusion. We conclude that a positive GVV-test before treatment makes a favourable response of IAT on proteinuria likely in patients with FSGS. If a reduction of proteinuria can be obtained by IAT this is due to an improvement in glomerular size and/or charge selectivity.

Key words: permeability factor; permselectivity; plasma immunadsorption; primary focal and segmental glomerulosclerosis; proteinuria

Introduction

Primary focal and segmental glomerulosclerosis (FSGS) is the most frequent histological diagnosis in adult patients with nephrotic kidney disease [1]. Although the glomerular epithelial cell is the common primary target, a variety of alterations can induce this pattern of injury and in some cases a factor increasing glomerular permeability has been identified [2,3].

Recently Godfrin et al. [4] developed an in vitro system (GVV-test) using isolated rat glomeruli to demonstrate the presence of this permeability factor (PF) in patients’ sera. Savin et al. [5] described this compound as binding to staphylococcal protein A (SPA) with high affinity and, accordingly Dantal and co-workers [6], were able to reduce proteinuria in patients with early recurrent FSGS after renal transplantation by an extracorporeal plasma immunadsorption treatment (IAT) using SPA-covered cartridges. As a reduction in proteinuria is mandatory in these patients if the prognosis is to be improved [7], the primary aim of our study was to investigate whether patients who are likely to respond to IAT, which is laborious and expensive, can be identified in vitro using the GVV-test prior to therapy.

In addition, we wanted to describe in more detail the effects of IAT on proteinuria. The leakage of plasma proteins into Bowman’s space is due to an alteration in glomerular permselectivity. The latter can be characterized experimentally by two distinct features (i) size selectivity, i.e. the ability of the glomerular filter to progressively hinder the passage of macromolecules of increasing molecular radius; and (ii) charge selectivity, the ability to restrict filtration of negatively charged molecules more effectively than that of equally sized uncharged or cationic compounds. The fractional clearance of neutral polyanisperse dextran molecules is used to create a sieving profile to describe size selectivity whereas negatively charged dextran sulfate is used to evaluate charge selectivity [8]. The integrity of the glomerular epithelial cell (GEC)
seems to be important for the maintenance of both filter components [9,10].

**Patients and methods**

All patients with native kidney FSGS were resistant to standard immunosuppressive therapy, including steroids, cyclophosphamide and cyclosporine A. Patients with recurrent FSGS in a transplant kidney were on triple-drug immunosuppressive therapy.

Patient 1 was a 49-year-old woman who started IAT 34 months after the histological diagnosis of FSGS.

Patient 2 was a 16-year-old girl. An initial biopsy performed because of massive nephrotic syndrome revealed minimal-change glomerulonephritis. A second biopsy was carried out 15 months later because of resistance to therapy and FSGS was diagnosed. IAT was started 3 weeks later.

Patient 3, a 23-year-old man, was also diagnosed initially with minimal-change glomerulonephritis. FSGS was the diagnosis after the second biopsy 14 months later and IAT was initiated 8 months thereafter.

Patient 4 was a 29-year-old man who started IAT 48 months after FSGS was diagnosed.

Patient 5 was a 25-year-old man with an 8-year medical history of proteinuria. He refused diagnostic work up until severe nephrotic syndrome developed after an upper respiratory tract infection. Biopsy revealed FSGS that was resistant to immunosuppressive treatment and IAT started 13 months later.

Patient 6 lost native kidney function due to FSGS and was on dialysis for 2 years. He received a cadaveric allograft and proteinuria developed within 4 weeks. A transplant biopsy carried out 4 weeks after engraftment established recurrent disease and after an additional 3 months IAT was started.

Patient 7 was a 29-year-old man who reached end stage renal failure 8 years after FSGS was diagnosed. After 2 years on dialysis he received a cadaveric kidney transplant with immediate recurrence of proteinuria. Several rejection episodes had to be treated before IAT was initiated 5 months later.

Patient 8, a 43-year-old man, was on haemodialysis for 2 months before he received a cadaveric renal allograft. Proteinuria developed within 3 weeks and a biopsy was finally performed after 5 months showing characteristic features of FSGS. IAT began 2 months later.

**Immunoadsorption procedures**

Six patients were treated with protein-A and 2 (Patients 5 and 8) with IgG immunoadsorption. After primary plasma separation with the autopheresis-CTM TPS (Therapeutic Plasma System, Baxter, IL) at each session, 2.5 plasma volumes were run at a flow rate of 35 ml/min over two columns containing 62.5 ml of SPA (Immuno-adsorb, Excorim, Sweden) or an anti-IgG antibody (1g-Therasorb, Munich, Germany). After plasma loading, the columns were washed with sodium citrate at a pH of 2.2 (SPA column) or with a 0.2 M glycine HCl buffer at a pH of 2.8 (IgG adsorption). Finally the columns were flushed with PBS (pH 7.2) and 0.9% NaCl solution. One cycle of therapy consisted of five treatment sessions within 10 days. If no response was obtained (defined as a reduction of proteinuria > 50%) the cycle was repeated after 1 week. However, patients resistant to one cycle remained unresponsive.

Clearance measurements and determination of the 24-h protein excretion were performed simultaneously 1 day before and 2 days after the first treatment cycle.

**Permselectivity studies**

Iothalamate (as a measure of glomerular filtration rate, GFR), neutral-dextran- and dextran sulfate-clearance studies were performed before and after IAT as described in detail elsewhere [8]. In summary the patients received an iothalamate bolus followed by a constant infusion. A dextran 40–dextran 70 mixture at a total dose of 130 mg dextran/kg bodyweight was used to determine size selectivity. Clearance values for dextrans up to a molecular radius of 58 Å could be analysed in all patients. A bolus of tritiated dextran sulphate was administered to quantify charge selectivity. The fractional clearance of each marker (Qm) was calculated as the clearance of each marker divided by the clearance of iothalamate. Higher fractional clearance values indicate an impairment of the respective permselectivity component.

**GVV studies**

The in vitro test for the presence of a factor altering glomerular albumin permeability in patient plasma was performed as described by Godfrin et al. [4,11]. Briefly glomeruli were isolated from male Wistar rats (Charles River, St Elbouef, France) and rinsed using an oxygenated isotonic R.P.M.I. 1640 solution (Gibco, Eragny, France) containing 6% BSA. The mean glomerular volume of approximately 1500 glomeruli was measured using a Multisizer counter (Coulter, Hileah, FL). Afterwards glomeruli were incubated with patient sera and then exposed to hypo-osmotic stress (1% BSA). The presence of the permeability factor increases albumin leakage out of the glomeruli and leads to a reduction in glomerular volume by concomitant water transfer. The glomerular volume variation (GVV) was defined as:

\[
\text{GVV} (%) = 1 - \left( \frac{\text{glomerular volume of test sample}}{\text{glomerular volume of control sample}} \right)
\]

According to Godfrin et al. [11] a variation in the glomerular volume exceeding 3% was considered significant. The test was carried out in triplicate using sera taken before and after IAT in every patient and the calculated mean was used for analysis.

Statistical analysis was carried out using Student’s t-test for paired data. All values are given as means ± 1 SEM. The study was approved by the local human subjects committee.

**Results**

As can be seen from Table 1, immunoadsorption therapy reduced serum IgG, IgM, IgA and immunoglobulin light chain concentrations in all patients. Individual data on GFR, 24-h urinary protein excretion and the values for the fractional protein excretion, the fractional clearance of 58 Å neutral dextran and dextran sulphate before and after the first cycle, are given in Table 2. Proteinuria was reduced by 50% or more in 4 patients (1, 5, 6 and 7). This decrease was not due to a reduction in serum protein concentrations by IAT as evidenced by a reduction in the fractional protein excretion.
Table 1. Serum immunoglobulin and light-chain concentration in patients with primary focal and segmental glomerulosclerosis before and after IAT

<table>
<thead>
<tr>
<th>Patients</th>
<th>IgG mg/100 ml</th>
<th>IgM mg/100 ml</th>
<th>IgA mg/100 ml</th>
<th>Igκ mg/100 ml</th>
<th>Igλ mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>1. W.M./NK</td>
<td>529 &lt;195</td>
<td>448 254</td>
<td>206 142</td>
<td>168 55</td>
<td>107 40</td>
</tr>
<tr>
<td>2. B.V./NK</td>
<td>350 &lt;195</td>
<td>169 74</td>
<td>169 68</td>
<td>116 &lt;40</td>
<td>73 &lt;25</td>
</tr>
<tr>
<td>3. L.R./NK</td>
<td>204 &lt;195</td>
<td>180 71</td>
<td>222 92</td>
<td>86 &lt;40</td>
<td>73 &lt;25</td>
</tr>
<tr>
<td>4. S.G./NK</td>
<td>345 &lt;195</td>
<td>134 &lt;30</td>
<td>483 44</td>
<td>194 &lt;40</td>
<td>134 &lt;25</td>
</tr>
<tr>
<td>5. L.H./NK</td>
<td>413 357</td>
<td>108 60</td>
<td>245 &lt;30</td>
<td>126 86</td>
<td>88 50</td>
</tr>
<tr>
<td>6. F.B./TX</td>
<td>582 &lt;195</td>
<td>103 &lt;30</td>
<td>142 69</td>
<td>157 43</td>
<td>97 30</td>
</tr>
<tr>
<td>7. G.W./TX</td>
<td>870 &lt;195</td>
<td>245 71</td>
<td>159 84</td>
<td>213 53</td>
<td>138 35</td>
</tr>
<tr>
<td>8. B.M./TX</td>
<td>611 &lt;195</td>
<td>179 98</td>
<td>192 118</td>
<td>167 56</td>
<td>112 39</td>
</tr>
</tbody>
</table>

Table 2. GFR, 24-h urinary protein excretion, fractional protein excretion, fractional 58 Å dextran- and dextransulfate-clearance and GVV in patients before and after IAT

<table>
<thead>
<tr>
<th>Patients</th>
<th>GFR (ml/min)</th>
<th>UProtV (g/d)</th>
<th>fr. Prot</th>
<th>Dex 58 Å</th>
<th>DS</th>
<th>GVV* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>1. WM NK PA</td>
<td>46 64</td>
<td>7 0.5</td>
<td>0.0008</td>
<td>0.0002</td>
<td>0.0033</td>
<td>0.0014</td>
</tr>
<tr>
<td>2. BV NK PA</td>
<td>18 20</td>
<td>29 26</td>
<td>0.0199</td>
<td>0.0195</td>
<td>0.0428</td>
<td>0.0409</td>
</tr>
<tr>
<td>3. LR NK PA</td>
<td>164 141</td>
<td>10 10</td>
<td>0.0011</td>
<td>0.0014</td>
<td>0.0057</td>
<td>0.0052</td>
</tr>
<tr>
<td>4. SG NK PA</td>
<td>84 39</td>
<td>13 19</td>
<td>0.0035</td>
<td>0.0035</td>
<td>0.0068</td>
<td>0.0066</td>
</tr>
<tr>
<td>5. LH IKG</td>
<td>43 56</td>
<td>9 4</td>
<td>0.0012</td>
<td>0.0006</td>
<td>0.0095</td>
<td>0.0013</td>
</tr>
<tr>
<td>6. FB TX PA</td>
<td>30 49</td>
<td>14 4</td>
<td>0.0026</td>
<td>0.0011</td>
<td>0.0182</td>
<td>0.0061</td>
</tr>
<tr>
<td>7. GW TX PA</td>
<td>32 40</td>
<td>6 3</td>
<td>0.0013</td>
<td>0.0008</td>
<td>0.0077</td>
<td>0.0007</td>
</tr>
<tr>
<td>8. BM TX IG</td>
<td>69 82</td>
<td>4 3</td>
<td>0.0004</td>
<td>0.0003</td>
<td>0.0125</td>
<td>0.0099</td>
</tr>
<tr>
<td>Mean</td>
<td>61 61</td>
<td>11 9</td>
<td>0.0039</td>
<td>0.0034</td>
<td>0.0133</td>
<td>0.0099</td>
</tr>
<tr>
<td>SEM</td>
<td>17 13</td>
<td>3 3</td>
<td>0.0023</td>
<td>0.0023</td>
<td>0.0045</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Dextran 58Å, fractional neutral-dextran-clearance at 58Å; DS, fractional dextransulfate clearance; GVV, glomerular volume variation; UProtV, 24-h urinary protein excretion; GFR, glomerular filtration rate; PA, protein A column; IG, IgG column.

Mean GFR and urinary protein excretion for all eight patients did not change significantly during the study (61 ± 17 ml/min vs 61 ± 13 ml/min and 11.5 ± 2.9 g/day vs 8.7 ± 3.3 g/day before and after IAT, respectively). Immunadsorption significantly reduced the mean fractional clearance of 58 Å neutral dextrans (Q_{DS} 0.0133 ±0.0045 before vs 0.009 ±0.0047 after therapy) mainly due to a dramatic improvement in glomerular size selectivity in responsive patients. The differences in fractional clearance values for smaller dextran molecules were not statistically significant between the two groups at any time. Charge selectivity improved in both patients with a positive pre-treatment GVV-test and in one additional patient; however, for the patient as a whole group no significant change in the fractional clearance of dextran sulfate was noted (Q_{DS} 0.68 ±0.073 before vs 0.54 ±0.046 after IAT). Two patients (5 and 7) had a positive and 6 a negative GVV-test before the first session of immunadsorption. When the test was carried out using sera obtained after the first treatment cycle GVV decreased to 0% in the previously positive patients and remained negative in the others (Table 2).

In patients responsive to IAT proteinuria relapsed after a mean of 21 days, at which time the treatment was repeated successfully in all but one patient. Patient 1 had been on IAT for 1.5 years when a stable remission of proteinuria was achieved. In patient 5, proteinuria increased 1 month after therapy and was then resistant to further intervention. Patient 7 lost his graft 3 months after the initiation of IAT due graft rejection. Patient 6 has now been treated for almost 2 years and his fractional protein excretion has been reduced by an order of magnitude.

Discussion

Primary FSGS is characterized clinically by nephrotic range proteinuria and histologically by solidification of the glomerular tuft and obliteration of the glomerular capillary lumen by a relatively acellular matrix material on light-microscopy, and by diffuse epithelial foot process fusion on electron-microscopy. The glomerular epithelial cell is considered to be the common primary target of injury and it is likely that a variety of different pathogenetic factors lead to the same histopathological pattern. It has been suggested that in some patients a circulating factor might be responsible for podocyte damage as early proteinuria after
ingraftment and the presence of an unknown factor, able to increase permselectivity for albumin, have been reported in several patients with recurrent FSGS following kidney transplantation [12]. The exact nature of this permeability factor is still a matter of intense research, however, an association with the immunglobulin protein family is likely as extracorporal IAT reduced proteinuria in kidney-transplant recipients with recurrent FSGS [6]. Unfortunately, immunadsorption therapy is laborious and extremely expensive and therefore in vitro tests that identify patients who are likely to respond to treatment are highly desirable. Savin et al. [5] and Godfrin et al. [4] incubated isolated rat glomeruli with sera from patients with FSGS. In the presence of the permeability factor, hypo-oncotic stress lead to a reduction in the glomerular volume, whereas without this factor no shrinkage in glomeruli was observed when exposed to 1% BSA. Accordingly Dantal et al. [11] showed a reduction in GVV after IAT in three patients with recurrent FSGS after transplantation. In our small patient population a positive pre-treatment GVV-test was highly predictive of a favourable response, however, a negative test did not completely eliminate the possibility of successful immu
nadsorption treatment. These data are in accordance with the report of Savin et al. [5]. In their population of 7 patients with native kidney FSGS a positive GVV-test predicted a decrease in proteinuria after treatment with plasma-separation. In addition they showed, that this permeability factor was present in only approximately 20% of patients with native kidney FSGS. Proteinuria in FSGS is the result of an alteration in glomerular permselective properties. For direct assess-ment of this function, exogenous, tubular inert markers are used. We employed the neutral-dextran-clearance technique to describe glomerular size selectivity, and dextran sulfate was used to determine charge selectivity. All patients responding to therapy showed an improve-
ment in glomerular size selectivity, and in three patients (including the ones with a positive GVV-test) charge selectivity also improved. The exact histological structural correlation for the various components of glomerular permeability is still unclear. However, the integrity of the podocyte seems to be important for both. In minimal-change glomerulonephritis an iso-
lated loss of charge selectivity has been described [13]. The ultrastructural correlation is the complete fusion of foot processes of the glomerular epithelial cells. As the podocyte cell membrane contains negatively charged elements fusion of foot processes might either result in or be the result of a decrease of anionic density which ultimately might lead to an impairment of charge selectivity.

Epithelial cells, however, can recover rapidly from an insult as shown in the hexadimethrin animal model [14] and the improvement in charge selectivity seen in our patients could be a result of the elimination of the circulating permeability factor.

Size selectivity has been attributed in part to the correct interaction between basement membrane and covering podocyte. Membrane areas denuded of epip-thelial cells are typical for FSGS-like injury and are likely candidates for the structural correlate to the loss of size selectivity described in these patients [15]. It has been argued that this detachment might be the result of a more intense injury than in minimal-change glomerulonephritids. An amelioration of the insult will therefore be compatible with a partial restoration of glomerular size selectivity.

In summary the response of patients with FSGS to immunadsorption therapy might be predictable by a positive in vitro GVV-test. If a reduction in proteinuria can be obtained by IAT this may be due to an improvement in both components of glomerular permselectivity, size and/charge selectivity.

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