COMPLEMENT ACTIVATION AND C1q BINDING ACTIVITY IN HAEMODIALYSIS

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Introduction

It has been proposed that hypoxia occurring during haemodialysis (HD) is related to complement activation by the dialysis membrane. Formation of the leucoagglutinin C5a produces neutrophil aggregates which embolise to the pulmonary vasculature leading to arteriovenous shunting, pulmonary oedema, and thus hypoxia [1]. Further evidence in favour of this hypothesis has been provided by animal studies using leucocyte aggregometry and microcinematography [2]. However, studies directed at measurement of complement components, particularly those attempting to demonstrate depletion of C3 and C4 during dialysis, have produced conflicting results. It has proved difficult to demonstrate a correlation between the degree of complement activation, neutropenia and hypoxia. We thus set out to evaluate a new sensitive assay for complement activation fragment C3d, as a direct means of assessing complement activation by haemodialysis; thus avoiding the pitfalls of attempting to demonstrate depletion of components which are in a state of dynamic equilibrium, and many of which function as acute phase reactants. We also assayed basic components C3 and C4 during dialysis, and measured C1q binding activity (ClqBA) in order to detect release, or formation of immune complexes (IC).

Patients and methods

Twenty patients, established on long-term HD were examined. All were in a stable condition with no evidence of any other illness likely to affect the parameters measured. All the patients were using cuprophane membrane based dialysers (six Kiil, eight Nephross and six Gambro). Arterial blood samples were taken prior to dialysis from the arterial needle, and during dialysis at 15, 60 and 120 minutes from a rubber cuff in the arterial line. Samples for complement component assay were collected into ice cool EDTA bottles, kept on ice, and spun at 3000 RPM in a refrigerated centrifuge at 4°C. One millilitre of supernatant plasma was removed and stored in liquid nitrogen until assay. Samples
for C1qBA were collected into glass tubes incubated at 37°C for thirty minutes (with protamine when necessary to allow clotting to occur) and spun at 3000 RPM. Serum was stored at -20°C until assay. Samples were also collected into heparinised syringes, for blood gas measurement on an Instrumentation Laboratory 613 Blood Gas Analyser.

C3 and C4 were measured by conventional nephelometric assays, and C3d by a new nephelometric assay. (Plasma samples were treated with 11 per cent polyethylene glycol, spun at 1500g, and the supernatant removed. Laser nephelometry was performed after the addition of anti C3d antibody and the results expressed in mg/dl.)

C1q binding activity was measured by standard technique.

Results

Hypoxia occurred during all 20 dialyses and was maximal at 60 minutes (mean ± SEM; 75.9 per cent (± 2.4) of pre-dialysis value). The results of complement component assays and C1qBA can be seen in Table I and Figures 1, 2 and 3.

Pre-dialysis C3d values were elevated and rose significantly on dialysis at 60 and 120 minutes (p < 0.01). C3 concentrations were subnormal and fell slightly during dialysis (the fall did not reach significance). C4 remained normal throughout dialysis, although once again a fall did occur which did not reach significance. C1q binding activity was normal pre-dialysis and rose significantly at 15, 60 and 120 minutes (p < 0.01). A control study performed on donor blood confirmed that heparin, at the concentrations present in our samples, had no effect on C1qBA, nor did the addition of protamine in order to promote clotting. Only when samples were heparinised to a high level (4 and 10 units per ml) did total failure of coagulation lead to an artifactual elevation of C1qBA (40 per cent), probably related to fibrinogen presence, (adequate protamine treatment of these samples abolished the artefact).

<table>
<thead>
<tr>
<th>Units</th>
<th>C3d</th>
<th>C3</th>
<th>C4</th>
<th>C1qBA</th>
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<tbody>
<tr>
<td></td>
<td>mg/L</td>
<td>gm/L</td>
<td>gm/L</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>1.1 (0.09)</td>
<td>1.2 (0.08)</td>
<td>1.6 (0.12)*</td>
<td>1.9 (0.04)*</td>
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<tr>
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<tr>
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<tr>
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<td>13.8 (1.9)</td>
<td>23.8 (1.7)*</td>
<td>30.2 (2.4)*</td>
<td>29.1 (1.9)*</td>
</tr>
</tbody>
</table>

* Significant rise, p < 0.01

Means ±SEM

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C3d DURING DIALYSIS

C3d mg/dl

MINUTES ON DIALYSIS

(UPPER LIMIT OF NORMAL 0.8 mg/dl)

Figure 1

C3 AND C4 DURING DIALYSIS

C3 & C4 gm/l

MINUTES ON DIALYSIS

C3 NORMAL RANGE 0.55 - 1.2 gm/l
C4 NORMAL RANGE 0.2 - 0.4 gm/l

Figure 2

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Discussion

C3d is an inactive complement fragment, released from the parent molecule, C3, following activation. It thus serves as a relatively stable marker of C3 activation via either the classical, or alternative pathways. The new nephelometric assay has the great advantage over standard radial immunodiffusion techniques in that it can measure accurately C3d within the normal range (0.5 to 0.8mg/dl), and thus provide a sensitive indicator of complement activation during haemodialysis. Measurement of this activation fragment is more reliable than assay of C3 or C4, or total haemolytic complement activity (CH50), concentrations of which may be affected by rates of production, release and consumption.

It is possible that complement activation during dialysis occurs via the classical or alternative pathway, or indeed both. However subnormal C3 values, with normal C4, suggest that a significant degree of activation occurs via the alternative pathway, most likely as a result of blood exposure to the polysaccharide cuprophane membrane. This results in chronic reduction in C3 and elevated pre-dialysis C3d values.

C1q binding activity is employed as a measurement of circulating immune complexes. Pre-dialysis values were normal in patients on chronic haemodialysis, but rose during dialysis. Our work to date, suggests that the rise in C1qBA reflects release of pre-formed immune complexes (IC) into the circulation during HD. These complexes may be displaced from anionic sites on the vascular endothelium.
by heparin. Alternatively, damage to the endothelium by reinfusion of activated blood components (platelets, leucocytes, etc.) [3] may dislodge pre-formed ICs during haemodialysis. Finally we have not, as yet, excluded the possibility that the ICs are formed de novo during haemodialysis, perhaps involving heparin, either as a stimulus, or as the antigenic component. Release of immune complexes may cause further activation of complement during dialysis, via the classical pathway by virtue of the complement fixing activity of the antibodies involved.

Activation of the immune system during dialysis may have implications beyond those of the probable association with neutropenia and hypoxia. Recurrent activation of complement, and immune complex release may be relevant to long-term complications of haemodialysis including endothelial damage (and accelerated atherogenesis), hypersplenism and thrombocytopenia.

References