HAEMODIALYSIS-INDUCED LEUCOPENIA AND ACTIVATION OF COMPLEMENT: EFFECTS OF DIFFERENT MEMBRANES


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Summary

The effects on neutrophil count and complement activity of five different haemodialysis membranes were studied. There was no correlation between the degree of neutropenia and intensity of complement activation. With cuprophan membrane both occurred simultaneously but to unrelated degrees; polyacronitrile induced mild, not significant neutropenia but marked activation of complement; polycarbonate membranes induced severe neutropenia without detectable complement activation. Where complement activation occurred it was via the alternative pathway. Haemodialysis induced neutropenia may have many causes and complement activation is probably not the major responsible factor.

Introduction

Patients undergoing haemodialysis experience a transient, profound and rapidly reversible leucopenia, mainly limited to neutrophils [1,2]. This neutropenia has been well documented with all types of cellophane membrane dialysers [3]. Clinical and experimental evidence suggests that the neutropenia arises from pulmonary sequestration of granulocytes [4]. The phenomenon can be reproduced by reinfusion of whole blood or plasma, but not albumin or saline, that has been in contact with cellophane membrane dialysers [5]. It has been postulated that a contact activated plasma factor is necessary to initiate the leucopenic effect of the dialyser [3,5].

Cellophane, like many other polysaccharides, is capable of inducing plasma complement activation [6]. Circulating activated components of the complement system have been detected during early haemodialysis [7]. These activated factors have been held responsible for the pulmonary leucostasis and resultant peripheral leucopenia observed in haemodialysis [7].

However, one study using anisotropic polysulphone membrane for haemofiltration, failed to demonstrate a marked leucopenia despite a significant fall
in complement levels [8]. Moreover previous work in our laboratory suggested a possible dissociation between activation of complement and leucopenia during polyacrylonitrile membrane haemodialysis [9]. These observations prompted us to undertake the present studies to determine the role of the complement system in relation to haemodialysis-induced neutropenia.

Material and Methods

Five groups of patients with end-stage renal failure undergoing routine haemodialysis were studied using five different types of dialysis membranes. None of them had clinical evidence of infection, was febrile or was taking drugs known to affect leucocyte count or complement. Details of the dialysis membranes, proportionating units, dialysers and number of patients are given in Table I.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Dialyser</th>
<th>Proportionating Unit</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Cuprophan (PM-150)</td>
<td>Meltec multipoint</td>
<td>Lucas Mk. II</td>
<td>10</td>
</tr>
<tr>
<td>Wet Cuprophan (PMW-200)</td>
<td>Meltec multipoint</td>
<td>Lucas Mk. II</td>
<td>7</td>
</tr>
<tr>
<td>Polyacrylonitrile (AN-69)</td>
<td>RP6</td>
<td>Rhodial 75</td>
<td>10</td>
</tr>
<tr>
<td>Dry Polycarbonate (Bard PCM dry)</td>
<td>Meltec multipoint</td>
<td>Lucas Mk. II</td>
<td>8</td>
</tr>
<tr>
<td>Wet Polycarbonate (Bard PCM wet)</td>
<td>Meltec multipoint</td>
<td>Lucas Mk. II</td>
<td>7</td>
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</tbody>
</table>

Dialysers were not re-used. Blood samples were withdrawn from the arterial line at -5, 0, 15, 30, 45, 60, 120, 180 and 240 minutes from the start of dialysis, for Coulter count, differential white cells count and complement assays.

Total complement levels were determined by the method of Kabat and Mayer with the modification of Fischer [10]. The results were defined in CH50 units (50% haemolysis point), using a calibration curve with serum from normal people. Interassay variations were corrected with the help of a constant standard serum.

The activity of the alternative pathway was measured by the haemolytic method described by Martin et al [11], expressing the sample activity as a percentage of the control. EGTA/Mg²⁺ was added to each sample in order to prevent classical pathway activation during the assay.

Factor B was determined by a radial immunodiffusion technique [11] using
a calibration curve prepared with a control serum and the results were given in percentage of this control.

C₃ and C₄ levels were measured by single immunodiffusion using C₃ and C₄ Behring antiserum respectively. As we did not find a significant change in C₃ and C₄ concentrations with this technique, additional samples were assayed by Laurell 'rocket' electrophoresis, and confirmed this observation.

The heparin content of all samples was insufficient to alter the reactivity of these assays, since the intravenous administration of 7,500 IU of heparin in five consecutive patients, not on haemodialysis, did not induce changes.

All results are expressed as percentage of pre-dialysis levels (mean value of 5 and 0 minute samples) and the overall results for each group as the mean ± SEM. A paired t-test was used to assess statistical significance.

Results

Patients using cuprophan dialysers developed a severe neutropenia within the first 45 minutes of haemodialysis. Considering the groups as a whole the nadir

Figure 1. Results in patients using dry cuprophan membrane dialysers (PM-150)
was at 15 minutes with dry cuprophan (26.2 ± 8.9% of the initial value; mean ± SEM; P < 0.001 — paired) and at 30 minutes (37.1 ± 6.2%; P < 0.001) with wet cuprophan. Simultaneously total haemolytic complement levels (CH50) decreased to 70.1 ± 6.2% (P < 0.005) with dry cuprophan and to 72.1 ± 6.4% (P < 0.005) with wet cuprophan (Figure 1).

During polyacrylonitrile membrane haemodialysis there was a slight reduction in neutrophil count at 15 minutes (89.2 ± 6.2%), which did not reach statistical significance. However, total complement levels decreased at 15 minutes to 75.1 ± 4.3% (P < 0.020) as did the alternative pathway activity and Factor B concentrations (P < 0.05) (Figure 2).

In all instances alternative pathway and Factor B changed in parallel with CH50. This is evidence that activation is mainly through the alternative pathway.

With PCM membranes there was a marked leucopenia, maximal at 15 minutes, being 45.3 ± 5.9% with dry PCM and 51.5 ± 6.2% with wet PCM (P < 0.001), but complement levels did not change significantly throughout the

Figure 2. Results in patients using polyacrylonitrile (AN-69) dialysers
Figure 3. Results obtained in patients using dry polycarbonate (Dry-PCM) dialysers

Figure 4. Overall changes in total complement activity (CH50) and neutrophil count in the five groups of patients
dialysis procedure, being 95.9 ± 2.0% with dry PCM and only 96.8 ± 2.3% with wet PCM at that time. There were no significant changes in alternative pathway and Factor B (Figure 3).

Figure 4 shows the overall results in each group of patients stressing the dissociation between intensity of complement activation and the degree of neutropenia.

Although there was a tendency for the C₃ and C₄ levels to fall, these changes were not significant. Nevertheless, the small drop was more consistent during cuprophan and polyacrylonitrile haemodialysis, where total complement (CH50) decreased significantly at the same time (Figure 5).

Discussion

Foreign surface leucopenia, as it is observed during haemodialysis, has been shown to be due to acute intravascular margination of neutrophils [2].
balance between the margined and circulating neutrophil pools depends on the intensity with which these cells adhere to the endothelium. In fact, during haemodialysis, there is a striking increase in granulocyte adherence, which is mediated by a plasma factor, reciprocally associated with the neutropenia and coinciding with the pulmonary leucocytosis [12].

From a series of relevant experiments Craddock and co-workers concluded that the adherence factors, in patients being dialysed, are complement products activated by cellophane membrane dialysers [7,13]. However, other investigators have recently challenged this theory by showing that the dialysis adherence factor is absent from serum, and is also heat-stable [12,14]. This important property of the adherence factor makes it unlikely that the activity factor is a genuine active complement component.

The present data show that the degree of dialysis neutropenia and activation of complement vary considerably with the type of membrane used. In patients using dry or wet cuprophan, complement activation occurred over the same time scale as the neutrophil change, and maximum neutropenia and maximum complement activation were achieved simultaneously. It is therefore tempting to speculate that complement activation is at least partly responsible for the neutropenia in this situation. However, there are two bits of evidence against this. When individual patients are considered there is no relationship between the intensity of activation of complement and induced neutropenia and in our patient with no detectable complement activity a profound consistent neutropenia still occurred when he was treated with cuprophan dialysers. Moreover Woodward and Brubaker [15] were unable to block cellophane-induced leucopenia with the complement inhibitor 5-hydroxy-indole-3-acetic acid.

In our studies of polycarbonate dialysers severe neutropenia occurred in the absence of significant activation of complement. This was most marked with wet PCM membrane. It is therefore unlikely that the neutropenia produced by this membrane is complement-induced. In contrast polycrylonitrile membrane produced a significant complement activation with only a mild leucopenia. A similar observation has previously been reported with polychlorinated membranes in studies in vitro [8].

Our findings strongly suggest that complement is not the major responsible factor for dialysis leucopenia. The two phenomena may be independent, though simultaneous, or complement activation may be the less important of two or more mechanisms of leucopenia. Other possible mechanisms have been suggested in the past. Leucopenia and sequestration of leucocytes in the lung have been described after the injection of intact leucocytes or disintegrated neutrophils, aqueous extract of neutrophils [16], lysozyme [17] and endotoxin [18]. Theoretically, these events could all happen during dialysis and in the experimental models developed for the study of dialysis-induced leucopenia.

References

1 Kaplow, LS and Goffinet, JA (1968) JAMA, 203, 1135
2 Brubaker, LH and Nolph, KD (1971) Blood, 38, 623
Open Discussion

KLINKMANN (Rostock) If you are going to dialyse a patient repeatedly with the same membrane you are always inducing changes in complement as well as in the neutrophils. You may speculate that you are influencing the immune status of the patient. Do you have any idea if this would influence the outcome of transplantation?

ALJAMA I cannot tell you anything about the long term effects of complement activation, but as far as the white cell count is concerned, patients on cuprophan dialysers show a significantly lower pre-dialysis total white cell count than patients treated with polyacrylonitrile membrane dialysers. Probably this is important for transplantation because it may well influence tolerance to the immunosuppressive therapy. But we do not know yet the effect of periodic complement activation.

KLINKMANN You do not exclude that not only transfusion but also repeated use of specific dialysis membranes may have some influence on the immune status of the patient.

ALJAMA I have no data on this.

JONES (London) We have been measuring gas changes during haemodialysis using a variety of membranes and I was interested in your findings with polyacrylonitrile because that is the one membrane with which we found that the hypoxaemia early in dialysis does not occur and I wonder if you had the opportunity of measuring blood gases in any of your patients?

ALJAMA Yes, we measured blood gases throughout dialysis at the same time.
that we took samples for neutrophils and we found that the drop in pO₂ is not related to the drop in neutrophil count. In fact in general throughout polyacrylonitrile haemodialysis and during cuprophan dialysis the drop in pO₂ is similar, despite very significant differences in neutrophil count.

IVANOVICH (Chicago) In addition to the blood gas changes we have also measured lung perfusion scans with conventional regenerated cellulose membranes and a cellulose acetate membrane. The cellulose acetate membrane did not give the neutropenia seen with regenerated cellulose or cuprophan, nor was there as profound a drop in blood pO₂ and the lung perfusion scan, which shows a 50% drop with regenerated cellulose membrane, was completely normal, as with the control pre-dialysis lung scan.

PAPADIMITRIOU (Thessaloniki) Do the platelets follow the same pattern as the white count during dialysis? Secondly, did you give to your patients anti-complement factors such as e-aminocaproic acid in some of your experiments? Finally, we have had the same experience as you with cuprophan membranes but in patients with lupus and very low complement we did not find any significant decrease in the number of polymorphs during dialysis.

ALJAMA We have no data about platelet count during dialysis. Answering your second question, our patient with undetectable total complement activity showed on seven occasions transient and profound neutropenia despite no complement activity at all and a low C₃ level. Woodward and Brubaker induced total inhibition of complement by giving 3-hydroxy-5-indole-methyl-acetic acid and they showed that leucopenia occurred, so I do not know how to explain your findings in patients with SLE.

WOODS (London) In our studies we did not actually measure leucocytes, but there is some evidence to suggest that prostacyclin does inhibit the procoagulant activity of leucocytes, and while this work from Newcastle does suggest that Craddock's work is now questionable with regard to the interrelationship between complement and leucocyte falls, it would be very interesting to know whether in fact the Newcastle group looked at the procoagulant action of leucocytes as well as just the fall of leucocyte numbers. This may explain to some degree the sequestration and the changes which occur in leucocyte numbers. We believe that prostacyclin, like PGE₁ would inhibit the lysozomal procoagulant activity of leucocytes.

WAUTERS (Lausanne) You mention that magnesium could activate the alternate pathway. Do you have some results concerning the magnesium levels in your patients?

ALJAMA No.

BURCK (Kiel) Coulter counter results have great statistical variation. Red cell counts can vary up to 20% plus or minus. Did you compare white cell counts in the Coulter-counter system with Neubauer chamber counts?

ALJAMA No, we did not, but in any case the differences during cuprophan dialysis are too big to be ascribed to experimental error.
STUMMVOLL (Vienna) We performed plasma infusion studies after circulating plasma through artificial kidneys. We found a leucopenia and complement activation but we did not find thrombocytopenia or thrombocyte aggregation so there seem to be different mechanisms working.

ALJAMA Yes, in fact all the features we are commenting on, neutropenia, thrombocytopenia, complement activation, hypoxaemia etc. seem to occur at the same time, however they may well be unrelated and possibly independent.