LEUCOPENIA, HYPOXIA AND COMPLEMENT ACTIVATION IN HAEMODIALYSIS. THREE UNRELATED PHENOMENA

S G de Vinuesa, M Resano, J Luño, C Gonzalez, G Barril, E Junco, F Valderrabano

Hospital Provincial, Madrid, Spain

Summary

Acute, transient leucopenia occurs in uraemic patients during the first minutes of haemodialysis, haemofiltration and ultrafiltration, and this leucopenia depends on the membrane used: maximal with cuprophan, less marked using cellulose acetate in haemofiltration and minimal with polycrylonitrile. Complement activation was noted in all dialysis procedures except ultrafiltration. However, no correlation was found between the intensity of the complement activation and the degree of leucopenia. Significant hypoxia only appeared in haemodialysis using an acetate bath even with the polycrylonitrile membrane. Sequential ultrafiltration-dialysis studies clearly demonstrate that leucopenia and hypoxia are unrelated effects of haemodialysis. Leucopenia depends on the membrane used and hypoxia may be related to the use of an acetate dialysate. In addition, the presence of dialysis fluid was necessary for membrane-induced complement activation suggesting an important influence of the dialysate on membrane biocompatibility.

Introduction

Transient leucopenia and hypoxia occurring during the first hour of haemodialysis, are well known phenomena [1]. Leucopenia mainly limited to neutrophils [2] results from pulmonary sequestration of leucocytes [3]. Whereas, Craddock et al suggested that complement activation by dialyser cuprophan was responsible for the leucopenia and pulmonary sequestration of neutrophils inducing a pulmonary dysfunction and hypoxia [1, 4, 5], other authors [6, 7] using membranes such as polysulphone or polycrylonitrile, failed to demonstrate a marked leucopenia despite significant complement activation. Additionally, hypoxia has been noted with dialysers that do not cause significant leucopenia [8, 9]. On the other hand, the report of Aurigemma [10] and our previous finding [11] demonstrating that bicarbonate dialysate prevents hypoxia during
dialysis prompted us to study prospectively the effects of acetate haemodialysis, bicarbonate haemodialysis, haemofiltration and ultrafiltration using different membranes on the complement system in relation to dialysis-induced leucopenia and hypoxia.

Material and methods

We studied 14 patients with end-stage renal failure undergoing chronic haemodialysis, aged 16–52 years, who had been on regular dialysis for at least 12 months. Eight of these patients were sequentially subjected, with an interval of 48–72 hours, to different dialysis procedures: 1) Haemodialysis with an acetate bath (39mEq/L) and a cuprophan dialyser (ALT-100, 1.08m²); 2) Haemodialysis with a bicarbonate bath (38mEq/L) and the same cuprophan dialyser; 3) Haemodialysis with acetate and a polyacrylonitrile dialyser (AN-69, 1.03m²) in a closed circuit (Rhodial-75 proportionating unit); 4) Haemofiltration performed with 20L infused in a post-dilution system (Sartorius) at an ultrafiltration rate of 60–100ml/min, using a cellulose acetate membrane (Sartorius haemofilter, 0.6m²), the replacement fluid contained 38mEq/L of acetate; 5) Ultrafiltration with the polyacrylonitrile membrane (AN-69).

Predialysis blood samples were drawn anaerobically from the arterial line and also at 15, 30, 60, 120, 180 and 240 minutes after initiating all the procedures except ultrafiltration in which the last sample was obtained at 120 minutes and immediately processed for leucocyte count (Coulter-S) and oxygen arterial tension (PaO₂, Technicon blood gases analyser). Blood samples for serum complement assays were allowed to clot at room temperature and sera were separated in aliquots and immediately frozen until analysed. C₃, C₄ and factor B were measured by radial immunodiffusion with monospecific antisera (Behring). Whole complement values were measured by two methods, CH₅₀ by immune-haemolysis using a microtitre plate assay and CH₄₅₀ by radial diffusion in an agarose gel medium which contained sheep red cells sensitised with haemolysin, standardised and immobilised (Quantiplate Kallestad Laboratories Inc, Austin, Texas, USA). Alternative pathway (AP) by the Quantiplate method (Kallestad Laboratories Inc) which standardises and immobilises the rabbit red cells in an agarose gel containing EDTA buffer.

The protocol of study in the other six patients consisted of one hour of ultrafiltration followed immediately by three hours of haemodialysis (sequential ultrafiltration-dialysis) using the polyacrylonitrile membrane and a Monitral (Hospal) dialysis monitor, in which the ultrafiltration predialysis process produces a mild hypothermia (31 to 33°C) at the dialyser outflow with rewarming to a normal blood temperature during dialysis. In these patients blood samples were obtained in the same manner and for the same determinations as the other group at 0, 15, 30 and 60 minutes of ultrafiltration and at 15, 30 and 60 minutes after connection of dialysis fluid.

Data are expressed as the mean ± SEM, and for leucocyte count and complement values the percentage of the predialysis value were used. Statistical analysis was performed by a paired t-test. A 'p' value less than 0.05 was considered significant.
Results

Figure 1 shows the changes in leucocyte count, PaO$_2$ and whole complement (CH$_{100}$) during 240 minutes of all dialysis procedures and 120 minutes of ultrafiltration. Using a cuprophan dialyser, we observed a marked leucopenia within the first 15 minutes of starting haemodialysis from 5480 ± 321 to 2100 ± 250 cells/mm$^3$ (p < 0.001) with an acetate bath, and a similar decrease in the leucocyte count from 5310 ± 285 to 1910 ± 207 cells/mm$^3$ (p < 0.001) was noted in bicarbonate dialysis with the cuprophan membrane. In haemofiltration with cellulose acetate membranes the fall in the leucocyte count at 15 minutes was less marked, from 5640 ± 178 to 4240 ± 142 cells/mm$^3$ (p < 0.01), and the
<table>
<thead>
<tr>
<th></th>
<th>Whole complement</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Factor B</th>
<th></th>
<th></th>
<th></th>
<th>AP_100</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH_{150}</td>
<td>CH_{100}</td>
<td></td>
<td></td>
<td>C_4</td>
<td></td>
<td>C_3</td>
<td></td>
<td>Factor B</td>
<td></td>
<td>AP_{100}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>240 min</td>
<td></td>
<td></td>
<td>15 min</td>
<td>240 min</td>
<td>15 min</td>
<td>240 min</td>
<td>15 min</td>
<td>240 min</td>
<td>15 min</td>
<td>240 min</td>
</tr>
<tr>
<td>Acetate haemodialysis (Cuprophan)</td>
<td>*</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>*</td>
<td>%</td>
<td>*</td>
<td>%</td>
<td>*</td>
<td>%</td>
<td>***</td>
<td>71±3.3</td>
</tr>
<tr>
<td>Bicarbonate haemodialysis (Cuprophan)</td>
<td>*</td>
<td>%</td>
<td>**</td>
<td>**</td>
<td></td>
<td>*</td>
<td></td>
<td>**</td>
<td></td>
<td>**</td>
<td>***</td>
<td>70±2.8</td>
</tr>
<tr>
<td>Acetate haemodialysis (Polyacrylonitrile)</td>
<td>***</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
<td>**</td>
<td>***</td>
<td>**</td>
<td></td>
<td>***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultradialfiltration (Polyacrylonitrile)</td>
<td>94±2.8</td>
<td>100±2.1</td>
<td>99±2</td>
<td>100±3.2</td>
<td>102±2</td>
<td>94±4.6</td>
<td>110±4.6</td>
<td>102±3.2</td>
<td>102±2.1</td>
<td>96±2</td>
<td>99±3.6</td>
<td></td>
</tr>
<tr>
<td>Haemofiltration (Cellulose acetate)</td>
<td>72.7±3.9</td>
<td>79±5.3</td>
<td>106±2</td>
<td>99±5</td>
<td>102±2</td>
<td>85±5</td>
<td>98±2</td>
<td>90±3.5</td>
<td>99±2</td>
<td>80±2.1</td>
<td>101±1.5</td>
<td></td>
</tr>
</tbody>
</table>

† The last sample in ultrafiltration was taken at 120 minutes

Results are given in percentage of predialysis values mean ± SEM. CH_{150}, were not measured at the end of the procedure.

* p < 0.05; ** p < 0.01; *** p < 0.005
polyacrylonitrile membrane during the first minutes of haemodialysis or ultrafiltration produced a mild but significant decrease from 5700 ± 245 to 4700 ± 178 cells/mm³ in haemodialysis and 5460 ± 168 to 4940 ± 198 during ultrafiltration (p < 0.05). This leucopenia was corrected in all procedures between 60 and 120 minutes of dialysis or ultrafiltration.
Arterial PaO₂ showed no significant differences with bicarbonate dialysis and increased during the first half hour of ultrafiltration. PaO₂ decreased progressively for two hours of acetate haemodialysis, and this hypoxia was not related to the membrane used. With the cuprophan dialyser, the PaO₂ fell from 91 ± 3 to 80 ± 4mmHg (p < 0.01) at 120 minutes, and from 92 ± 2 to 83 ± 3mmHg using the polyacrylonitrile membrane at the same time (p < 0.05). Although there was a clear tendency for decrease in the PaO₂ during the first hour of haemofiltration, the changes were not significant.

Determination of complement showed a significant decrease of whole complement, alternative pathway, C₃ and factor B, without any variation of C₄, at 15 minutes of dialysis, when compared with predialysis values in haemodialysis with cuprophan and with polyacrylonitrile membranes. A similar drop of the complement titres was observed in bicarbonate dialysis and in haemofiltration using a cellulose acetate membrane. However, in contrast to haemodialysis, ultrafiltration with a polyacrylonitrile membrane does not produce any significant changes in complement. All the early changes of complement were transient and reversible within two hours of the dialysis procedure (Table I).

The data obtained during sequential ultrafiltration and dialysis (Figure 2) confirmed that during the first 15 minutes of ultrafiltration, the polyacrylonitrile membrane caused a mild leucopenia from 4933 ± 321 to 4343 ± 350 cells/mm³ which resolved within one hour of ultrafiltration, there was no other significant change in the leucocyte count during the latter three hours of haemodialysis. However, the changes of the whole complement and C₃ were not significant during the first hour of ultrafiltration, but the connection of the dialysis fluid caused a marked decrease of CH₅₀ (84 ± 3%, p < 0.01) and C₃ (77 ± 3.7%, p < 0.01) at 15 minutes after initiating haemodialysis. The changes of factor B were not significant. Although there was a significant decrease of alternative pathway with ultrafiltration, this fall was more marked when the dialysate was connected. PaO₂ values, which increased slightly during ultrafiltration, fell during the first 15 minutes of haemodialysis from 94.7 ± 5 to 84.3 ± 3.5mmHg, p < 0.05.

Discussion

The present work using different membranes in various dialysis procedures confirms the fact that a transient leucopenia occurs in all patients being dialysed [2, 12]. This leucopenia occurs early in all dialysis procedures (haemodialysis, haemofiltration and ultrafiltration) but the degree of leucocyte count fall depends on the membrane used and seems not to be related to the dialysis procedure. It is maximal with cuprophan, less marked with cellulose acetate in haemofiltration and minimal using polyacrylonitrile membrane in haemodialysis or ultrafiltration. In spite of these differences, the intensity of complement activation, mainly through alternative pathway, which occurs simultaneously with leucopenia in haemodialysis (using polyacrylonitrile or cuprophan) and haemofiltration, is similar. Indeed, we could note a dissociation between these two phenomena in haemodialysis with polyacrylonitrile when minimal leucopenia occurs despite a significant complement drop. An important observation
in our work is that in contrast to regular haemodialysis with polyacrylonitrile, ultrafiltration alone with the same membrane causes no important changes in serum complement values, but is associated with the same degree of mild leucopenia. This is in contrast to the report of Craddock [4] who suggests that membrane-induced complement activation is responsible for pulmonary leucostasis and leucopenia. Their conclusion cannot be supported by our results using the polyacrylonitrile membrane in ultrafiltration, where leucopenia occurred in the absence of significant complement activation. It is, therefore, unlikely that at least with the polyacrylonitrile membrane, the leucopenia was complement-induced.

In a study with polysulphone membrane Henderson et al could not demonstrate a significant leucopenia despite evidence of complement activation [6] and Aljama et al using cuprophan, polyacrylonitrile and polycarbonate membranes for haemodialysis noted that leucopenia and complement activation may be simultaneous but not related [7]. There is no clear explanation for the absence of significant activation of the complement system during ultrafiltration. This may result from inability of the polyacrylonitrile membrane to activate complement in the absence of dialysis fluid. During ultrafiltration in vivo the ultrafiltration membrane as a consequence of the convective transport, becomes coated with a protein layer which may block its complement-activating capacity. It is known that re-use of a dialyser enhances its biocompatibility, possibly by a similar mechanism, unless the re-used dialyser is rinsed with isotonic saline [13]. This theory is consistent with our data in sequential ultrafiltration-haemodialysis, showing a marked decrease of the complement values only after connection of the dialysis fluid. Understanding of this phenomenon is further complicated by failure of preventing complement activation in haemofiltration, a convective process by which water and solutes are also removed by ultrafiltration. This complement activation in haemofiltration using a cellulose acetate membrane, in contrast to ultrafiltration, may result from different properties of the membrane used. On the other hand, during haemofiltration the blood flow needed to obtain massive ultrafiltration up to four litres per hour, was markedly higher than during ultrafiltration alone. It is known that increasing blood flow through the dialyser reduces protein aposition [14], which may affect the complement activating capacity of the membrane.

The present work shows that hypoxia only occurs during acetate haemodialysis, independent of the membrane used and progresses for two hours even as the leucocyte count and complement values return to normal. Moreover, the finding of increased PaO₂ with ultrafiltration and the absence of hypoxia during bicarbonate haemodialysis suggest that leucopenia and hypoxia may be simultaneous but unrelated events [15] and tend to negate the role of complement activation as the main factor responsible for dialysis-induced hypoxia. Thus, the suggested sequence of complement mediated pulmonary leucostasis leading to leucopenia and hypoxia [1] may not be valid. Another possible cause of this hypoxia is hypoventilation secondary to CO₂ loss [16, 17]. However, the rate of CO₂ loss compared with CO₂ production is minor and should not significantly depress respiration [19]. The mechanism of CO₂ loss during acetate haemodialy-
sis, the consumption of CO₂ during acetate metabolism, may depress respiration and decrease arterial pO₂ [18, 19].

The clinical implications of these three biological haemodialysis phenomena are not known and further studies in this area are required.

References

9. Dumerle F, Levin NW. Arch Intern Med 1979; 139: 1103

Address for correspondence: J Luño, Hospital Provincial, Madrid, Spain

Open Discussion

KERR (Newcastle) Congratulations on a very interesting and very thorough study. First a comment and then a question. I am very intrigued by your failure to activate complement during ultrafiltration and the subsequent activation during haemodialysis, I think it is unlikely to be explained by different rates of ultrafiltration. When we have studied the effect of re-use, once a protein film is formed (if that is what the explanation of the effect of re-use is) it is quite difficult to remove. It needs vigorous washing or hypochlorite to get rid of it so it is a little surprising if it disappears just because you reduce the rate of ultrafiltration. It would be even more convincing if you had demonstrated no complement activation during ultrafiltration with a Cuprophan membrane. Have you tried substituting Cuprophan for Polycrylonitrile to see if you still get no complement activation?

LUÑO We are now trying to measure complement activation during ultrafiltration with Cuprophan, but we have no data yet.
VALENTYN (Leiden) I am not convinced that you actually demonstrated complement activation and not just depressed values. For activation you have to demonstrate C₃ breakdown products. Do you have data of such measurements?

LUÑO No, we have not measured C₃ breakdown products, but other authors have found C₃b early in dialysis. Nevertheless it is difficult to explain the fall in complement titres during dialysis procedures by any vascular volume changes or membrane absorption. This phenomenon occurs early during the first minutes of each dialysis schedule and in all procedures we get the same ultrafiltration volume (between 3—4 per cent of the body weight). Additionally the complement drop is a transient event which is reversible within an hour of dialysis. The fall in all complement titres except C₃ suggests complement activation mainly through the alternative pathway.