Effect of permeability on indices of haemodialysis membrane biocompatibility

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Abstract

Background. Increases in plasma anaphylatoxins frequently are used as an index of haemodialysis membrane biocompatibility; however, their plasma levels may be influenced by the loss of anaphylatoxins into the dialysate compartment.

Methods. We compared the generation and compartmental distribution of anaphylatoxins, C3a and C5a, in a high flux and a low flux polysulfone membrane dialyser when whole human blood was recirculated through an in vitro haemodialysis circuit.

Results. Plasma C3a levels in high flux polysulfone (2.31 ± 0.81 mg/ml) and low flux polysulfone (3.02 ± 0.98 µg/ml) dialysers were comparable after 120 min (P = NS). In contrast, dialysate C3a in high flux polysulfone (0.65 ± 0.31 µg/ml) accounted for 37.5 ± 7.0% of the total detected (plasma + dialysate) C3a mass in the dialysers, while dialysate C3a in low flux polysulfone dialysers (0.01 ± 0.01 µg/ml) accounted for only 0.3 ± 0.3% of the total mass (P < 0.05; high flux vs low flux). Anaphylatoxin C5a was undetectable in the dialysate compartment of either dialyser examined.

Conclusions. Our results indicate that anaphylatoxins readily traverse certain high flux dialysis membranes; consequently, plasma C3a levels may not accurately reflect the C3-activating potential of these membranes.

Key words: anaphylatoxins; biocompatibility; complement; haemodialysis; membranes; permeability

Introduction

Current evidence suggests that biocompatibility characteristics and solute clearance of dialysis membranes influence the long-term clinical outcome of chronic haemodialysis patients [1,2]. Complement activation occurs almost universally during clinical haemodialysis with the consequential generation of several complement activation products [3–5]. One of these products, anaphylatoxin C3a, is cleaved from the third component of complement during its activation [6]. Another anaphylatoxin, C5a, a fragment of the fifth component of complement, is generated as a result of C5 activation [6]. Once formed, C3a and C5a are converted rapidly to their respective desarginine derivatives, C3a_desArg and C5a_desArg, by a carboxypeptidase in plasma. The plasma levels of C3a and C5a_desArg during haemodialysis commonly are used as an index of dialysis membrane biocompatibility [3–5]. Plasma C5a and C5a_desArg are used less frequently for this purpose, probably because intradialytic activation of C5 is less pronounced and the increase in plasma levels of C5a and C5a_desArg is more modest [3,7].

Dialysis membranes are often classified as either high flux or low flux based on their ultrafiltration coefficient [8]. High flux membranes have larger pores and tend to clear middle molecules, such as β2-microglobulin, more efficiently, compared with low flux membranes [9,10]. Since C3a (Mr ~ 9 kDa) and C5a (Mr ~ 11 kDa) have molecular weights similar to that of β2-microglobulin (Mr ~ 12 kDa), it is likely that these complement fragments can also be transported across high flux membranes. If the loss of anaphylatoxins to the dialysate compartment is substantial, their plasma levels would underestimate the degree of complement activation by the dialysis membrane, similar to membranes that adsorb anaphylatoxins [11–13].

We wish to address the question of whether anaphylatoxins are in fact transported from plasma to the dialysate compartment and if this loss of anaphylatoxins significantly changes their plasma levels. To this end, we have compared the generation and compartmental distribution of C3a and C5a within a low flux dialyser with those in a high flux dialyser, both of which are made from the same material (polysulfone) by the same manufacturer. In addition, we compared these polysulfone dialysers with two other types of membranes that theoretically prohibit anaphylatoxin transport. One of these is Cuprophan® membrane which, despite its high capacity to activate complement, contains small pores. The other is AN69® membrane,
made from a co-polymer of acrylonitrile and methallyl sulfonate, which is known to adsorb anaphylatoxins [11–13].

**Subjects and methods**

**Haemodialysers**

The four different types of commercial hollow fibre membrane dialyser employed were (i) high flux polysulfone (F60, Fresenius, Concord, CA); (ii) low flux polysulfone (F6, Fresenius); (iii) Cuprophan® (2308, Travenol, Deerfield, IL); and (iv) AN69® (Filtral 12, Hospal, East Brunswick, NJ). Some of the characteristics of these dialysers are listed in Table 1. All four types of membranes have identical nominal surface areas. The two types of polysulfone membranes with different porosities were the primary focus of this study.

**Blood**

Six healthy human volunteers donated blood for the dialyser studies. Each volunteer donated blood on four separate days for experiments on each of the four types of membranes performed in random order. For each experiment, 200 ml of fresh blood was obtained by needle puncture of an antecubital vein of the volunteer. The blood was collected in porcine unfractionated sodium heparin (Elkins-Sinn, Cherry Hill, NJ) at a final concentration of 2 U/ml under sterile conditions, and used in experiments within 15 min.

**Simulated haemodialysis circuit**

The *in vitro* haemodialysis circuit was assembled as shown in Figure 1. Prior to use, the blood compartment of the dialyser was rinsed with sterile normal saline, which subsequently was removed with an air rinse. The heparinized blood (200 ml) for the dialyser blood compartment was maintained at 37 °C in a beaker which was partially immersed in a water bath. Commercial dialysate solution for the dialysate compartment, containing 137 mEq/l Na⁺, 3.0 mEq/l K⁺, 3.0 mEq/l Ca²⁺, 0.75 mEq/l Mg²⁺, 1.07 mEq/l Cl⁻, 4.0 mEq/l acetate, 33 mEq/l bicarbonate and 200 mg/dl dextrose, was freshly prepared using a Century 3 dialysis zero. All results are presented as mean ± SE. Levels of anaphylatoxins at various time points were evaluated using a non-parametric paired sign test. P-values <0.05 are considered to be statistically significant.

**Table 1. Characteristics of study dialysers**

<table>
<thead>
<tr>
<th>Material</th>
<th>Flux</th>
<th>Manufacturer</th>
<th>Model</th>
<th>Nominal surface area (m²)</th>
<th>Nominal ultrafiltration rate (ml/h/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysulfone</td>
<td>High</td>
<td>Fresenius</td>
<td>F60</td>
<td>1.3</td>
<td>30.8</td>
</tr>
<tr>
<td>Polysulfone</td>
<td>Low</td>
<td>Fresenius</td>
<td>F6</td>
<td>1.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Cuprophan®</td>
<td>Low</td>
<td>Travenol</td>
<td>2308</td>
<td>1.3</td>
<td>3.6</td>
</tr>
<tr>
<td>AN69®</td>
<td>High</td>
<td>Hospal</td>
<td>Filtral 12</td>
<td>1.3</td>
<td>23.9</td>
</tr>
</tbody>
</table>

Data taken from ref. 22.
Fig. 1. Schematic diagram of the *in vitro* dialysis circuit. Heparinized blood in a waterbath maintained at 37°C was recirculated through the blood compartment of a dialyser using a pump at a flow rate of 200 ml/min. Dialysate was recirculated in the closed dialysate compartment using a pump at a flow rate of 60 ml/min.

**Results**

*Generation and compartmentalization of C3a*

_Dialysis tubings alone_. Plasma C3a levels increased (*P*<0.01) gradually during recirculation through dialysis tubings alone. An apparent plateau phase was reached at 60 min when the mean C3a level was 1.63 µg/ml (not shown).

_Polysulfone membrane dialysers_. Plasma C3a levels increased (*P*<0.01) gradually during recirculation through either high flux (Figure 2A) or low flux (Figure 2B) polysulfone dialysers, reaching an apparent plateau at 60–90 min. At the end of the 120 min experiment, plasma C3a levels for high flux polysulfone (2.31±0.81 µg/ml) were comparable with those for low flux polysulfone (3.02±0.98 µg/ml), and were higher than those for tubings alone (mean 1.56 µg/ml). The C3a concentration in the dialysate compartment of high flux polysulfone dialysers at 120 min was 0.65±0.31 µg/ml, which was substantially higher than that found in the dialysate compartment of low flux polysulfone dialysers at the same time point (0.01±0.01 µg/ml) (Table 2). The total mass of C3a in the dialyser was calculated as (concentration × volume of plasma) + (concentration × volume of dialysate); plasma volume was calculated assuming a haematocrit value of 45% in the blood sample. The total C3a mass in high flux polysulfone dialysers (412±152 µg) was greater than that in low flux polysulfone dialysers (304±99 µg) in five out of six experiments, but the difference was not statistically significant. The C3a mass in the dialysate compartment accounted for 37.5±7.0% of the total mass in high flux polysulfone dialysers, but only 0.3±0.3% of the total mass in low flux polysulfone dialysers. It should be noted that, in these experiments, the C3a which might have been adsorbed onto the dialysis membrane was not quantified.

_Cuprophan® membrane dialysers_. As expected, plasma C3a levels increased (*P*<0.01) rapidly during blood recirculation through Cuprophan® membrane dialysers. The level reached a plateau phase at 60 min (Figure 3A). C3a was detectable in the dialysate compartment of Cuprophan® dialysers at 120 min, but the concentration was very low (0.16±0.07 µg/ml) and the mass in the dialysate compartment accounted for only 0.5±0.2% of the total mass in these dialysers (Table 2). Adsorption of C3a onto these membranes was not quantified.

_AN69® membrane dialysers_. Plasma C3a levels were low in the AN69® dialysers and remained stable throughout the recirculation (Figure 2C). At 120 min, plasma C3a level was 0.58±0.06 µg/ml, which equaled 37, 25, 19 and 1% of the corresponding plasma levels for tubings alone, high flux polysulfone, low flux polysulfone and Cuprophan® dialysers, respectively (Table 2). C3a was undetectable in the dialysate compartment of AN69® membrane dialysers.

*Generation and compartmentalization of C5a*

_Cuprophan® membrane dialysers_. Plasma C5a levels increased (*P*<0.0001) continuously during recirculation through Cuprophan® membrane dialysers and reached 49±9 ng/ml at 120 min (Figure 3B). This pattern was different from that of plasma C3a for the same type of dialyser (Figure 3A). No C5a was detected in the dialysate compartment at any time.

_Polysulfone and AN69® membrane dialysers and tubings alone_. There was no increase in plasma C5a levels during recirculation through tubings alone, high flux polysulfone, low flux polysulfone or AN69® membrane dialysers (data not shown). C5a was undetectable in the dialysate compartment of all these dialysers at any time points.
Fig. 2. Plasma (●) and dialysate (○) C3a concentrations during recirculation through high flux polysulfone membrane dialysers (A), low flux polysulfone membrane dialysers (B) or AN69® membrane dialysers (C).

Table 2. Distribution of C3a and C5a in the plasma and dialysate compartments of various dialysers

<table>
<thead>
<tr>
<th>Dialysers</th>
<th>C3a concentration (µg/ml)</th>
<th>C5a concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Dialysate</td>
</tr>
<tr>
<td>High flux polysulfone</td>
<td>2.31 ± 0.81</td>
<td>0.65 ± 0.31</td>
</tr>
<tr>
<td>Low flux polysulfone</td>
<td>3.02 ± 0.98</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Cuprophan®</td>
<td>45.25 ± 14.09</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>AN69®</td>
<td>0.58 ± 0.06</td>
<td>ND</td>
</tr>
<tr>
<td>Tubings alone</td>
<td>1.56</td>
<td>NA</td>
</tr>
</tbody>
</table>

*C3a and C5a concentrations at 120 min are presented as mean ± S.E. (n=6) for each type of dialyser and as mean (n=2) for tubings alone. Note that the anaphylatoxin levels associated with each dialyser resulted from the effect of the combination of the dialyser and tubings. ND = not detectable, i.e. below the lower detection limit for the C3a assay (0.04 µg/ml) and C5a assay (0.01 µg/ml) respectively. NA = not applicable because there was no dialyser and dialysate.

Discussion

Some recent studies have suggested that the type of dialysis membrane employed influences patient survival in both acute [14,15] and chronic [1] renal failure. The mechanism by which dialysis membrane exerts its effect is unclear, but the various degrees to which different membranes activate complement, neutrophils and monocytes have been suggested to be a factor. Anaphylatoxin C3a has been used commonly as a marker of complement activation during haemodialysis. While both anaphylatoxins are spasmogenic, the leukocyte-directed activities of C5a are greater than those of C3a [16]. However, the relatively low plasma level of C5a, partly as a result of its binding to specific receptors on leukocyte surfaces [17], has often been cited as a rationale for using C3a instead of C5a as a marker of complement activation. Other activation
products of C3, such as C3b and iC3b (both with \( M_r \sim 180 \text{ kDa} \)), can also bind to cytoplasmic membrane receptors (e.g. CR1 and CR3, respectively) and modulate leukocyte function [18]. Under favourable conditions, activation of C3 and C5 is followed by activation of the terminal components of complement and formation of the membrane attack complex, C5b-9 (\( M_r \sim 1500 \text{ kDa} \)); this complex has also been shown to be functionally active against leukocytes [5].

The activation products, C3b, iC3b and C5b-9, are seldom measured for the study of dialysis membrane biocompatibility, partly because their assays are not widely available, and partly because their plasma levels do not increase as drastically as C3a does. In essence, C3a is used commonly as an index of complement activation during haemodialysis, not necessarily because of its biological activity, but because of the magnitude of the intradialytic increase in its plasma concentration and the convenience of its assay. If C3a is lost from the blood compartment during haemodialysis, but the other biologically active complement activation products are retained, plasma C3a would not provide an accurate indicator of complement activation. Therefore, a more complete profile of complement activation by dialysis membranes should include the measurements of C3a, C3b, iC3b, C5a and C5b-9.

We have shown previously that the AN69® membrane has a large capacity to adsorb C3a, C5a and cytochrome c [11], thus prohibiting the transport of these molecules into the dialysate compartment despite the high porosity of this membrane. The absence of anaphylatoxins in the dialysate compartment of the AN69® membrane dialysers was confirmed in the present study (Figure 2C).

The other mechanism by which anaphylatoxins can be lost from the blood compartment is transport into the dialysate compartment. This was demonstrated in the present study for high flux polysulfone (F60) dialysers (Figure 2A). The C3a in the dialysate compartment accounted for almost 40% of the total mass in these dialysers. It should be noted that, in these experiments, the volume of the recirculating dialysate was small (280 ml or only 2.5 times the plasma volume); in addition, there was no net ultrafiltration. During clinical haemodialysis, where dialysate usually flows at 500–1000 ml/min in a single pass fashion, the concentration gradient between the plasma and dialysate, hence the diffusive loss of solutes, may be greater than those in the present study. In addition, net ultrafiltration of plasma water contributes to solute loss by convection. Therefore, the loss of C3a from plasma during clinical dialysis is expected to be greater than that observed in the present experiments.

Adsorption of C3a onto the dialysis membranes was not examined in the present study. It is unlikely, however, that the adsorptive capacity of low flux polysulfone membranes is higher than that of high flux polysulfone membranes with the same nominal luminal surface area. It thus appears that high flux polysulfone membranes activate complement at least to the same extent, or perhaps even to a greater extent, than low flux polysulfone membranes.

In accordance with its known property as a potent activator of complement, there was a large increase in plasma C3a levels associated with Cuprophan® dialysers during blood recirculation. Plasma C3a levels reached a plateau of 45–55 \( \mu \text{g/ml} \) after 60 min, in contrast to a continuous increase in plasma C5a levels reaching \( \sim 50 \) ng/ml at the end of the 120 min experiment. This discrepancy can be explained readily by the relative efficiency of C3 activation, such that all the plasma C3 (\( \sim 1 \) mg/ml) had already been converted into C3a (\( M_r = 5% \) of that of C3) at 60 min. In contrast, the formation of C5 convertase was relative inefficient, such that only a small fraction (<2%) of the total plasma C5 pool had been activated even at 120 min. The in vitro time course of plasma C3a changes described herein also differs from that observed during clinical dialysis using the same type of membrane. During clinical haemodialysis, plasma C3a levels achieve peak values at 10–20 min and decline there-
Anaphylatoxins and dialysis membranes

References


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