Dialyser re-use, neutropenia and complement activation

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REUTILIZACION DE DIALIZADORES, NEUTROPENIA Y ACTIVACION DEL COMPLEMENTO

RESUMEN

El objetivo de este trabajo fue valorar los efectos de la reutilización de dializadores sobre la neutropenia y la activación del complemento. Se estudiaron 12 pacientes que fueron tratados secuencialmente con diferentes membranas: cuprofán (CU), acetato de celulosa (CA), polisulfona (PS), policarbonato (PC) y poliacrilonitrilo (PAN). Con todos los dializadores se observó la aparición de una neutropenia significativa durante el «primer uso», que fue máxima a los cinco minutos para el CA, PS y PAN, y a los quince minutos para el resto de las membranas. El procedimiento empleado para la reutilización de los dializadores consistió en ultrafiltración inversa con agua corriente durante veinte minutos y almacenamiento con formaldehído al 3 %. El grado de neutropenia fue menor cuando los pacientes fueron dializados con membranas de CU reutilizadas; sin embargo, no se encontraron modificaciones de este parámetro con el resto de los dializadores. La capacidad de activación del complemento fue determinada por la medición de los niveles plasmáticos de C3d. Los valores más elevados de C3d coincidieron simultáneamente en el tiempo con la máxima leucopenia, excepto con el PAN. Sólo se encontraron diferencias significativas en la activación del complemento con el CU reutilizado, permaneciendo estables en las otras membranas. Nuestros resultados indican que la reutilización de dializadores mejora el grado de biocompatibilidad únicamente en las membranas de CU, no observándose cambios en la celulosa modificada ni en las membranas sintéticas.

Palabras clave: ***Biocompatibilidad. Complemento. Hemodiálisis. Leucopenia. Reutilización.***

SUMMARY

The effect of re-use on neutropenia and complement activation was investigated in 12 patients successively dialyzed on cuprophan (CU), cellulose acetate (CA), polysulfone (PS), polycarbonate (PC) and polyacrilonitrile (PAN). A significant neutropenia occured on every membrane during their first use, maximal at 5 min for CA, PS and PAN and at 15 min for the other membranes. Reprocessing of dialyzers consisting of reverse ultrafiltration and formaline 3 % storage improved neutropenia only on CU. Complement activation as measured by C_{3d} accumulation was observed on all the membranes at the maximum neutropenia, except on PAN. Reprocessing produced improvement of C_{3d} values on CU only. Our data indicate that the re-use of dializers improves the biocompatibility of CU, but not of modified cellulose and synthetic membranes.

Key words: ***Biocompatibility. Complement. Hemodialisis. Leucopenia. Reuse.***

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Introduction

It is now well established that the marked neutropenia observed during the initial phase of haemodialysis with cellulosic membranes results from pulmonary sequestration of activated neutrophils, as a consequence of dialyzer-associated activation of the complement system through the alternative pathway [1-3]. This series of events lead to the generation of anaphylatoxins C_{3a} and C_{5a} and they have been related to the pulmonary dysfunction and hypoxemia occasionnally observed during the procedure [4, 5]. Doubt has been cast upon the role of complement in dialysis-mediated neutropenia when polyacrilonitrile membranes were found to activate complement with only mild neutropenia and polycarbonate membranes failed to activate complement, but induced neutropenia [6, 7]. This problem has been partly explained by the fact that polyacrilonitrile membranes adsorb a large variety of substances including C_{5a} [8].

Complement activation has been demonstrated by bioassays based on leukocyte aggregometry [9] and by radioimmunoassays for C_{5a} and C_{3a} [10, 11]. The short half-life of C_{5a} and its rapid binding to the neutrophil surface receptors [12] makes it a less sensitive marker than C_{3a} which seems to remain free in plasma [13]. More recently measurement of the C_3-derived C_{3d} fragment has been shown to provide a convenient and specific cumulative market of complement activation [14, 15].

In recent years, the development of new dialyzers has led to the appearance of numerous reports comparing the leukopenia and complement-activating potential of these new membranes. Non cellulosic membranes have been shown to provoke less neutropenia and correspondingly complement activation [10, 11, 16-18]. That re-use ameliorates leukopenia induced by cuprophan has been repeatedly demonstrated [19-23], but only few studies have been devoted to the re-use of synthetic membranes [21].

Therefore, the effect of re-use on neutropenia and complement activation was investigated in a prospective cross-over study undertaken in 12 patients successively dialyzed on cuprophan, cellulose acetate, polysulfone, polycarbonate and polyacrilonitrile.

Methods

Patients, dialyzers and re-use procedures

Twelve patients, 7 females and 5 males, from 47 to 80 years old, voluntarily participated in these studies; all patients had been dialyzed for 2 to 109 months with a mean duration of 32 months. They were treated for three hours three times weekly as already described [23].

All 12 patients began the study with a cuprophan dialyzer (CU), which was their usual membrane (CF 1511; Travenol, USA; surface area 1,3 m^2). At each subsequent month the following dialyzers were tested: cellulose acetate (CA) dialyzer (CA 170; Travenol, USA; surface area 1,7 m^2), polysulfone (PS) (F 60, Fresenius, Germany; surface area 1,3 m^2), polycarbonate (PC) (Lundia Pro 5; Gambro Lundia, Sweden; surface area 1,1 m^2) and finally polyacrilonitrile (PAN) (Biospal 2400 S; Hospal France; surface area 1,0 m^2). In the intervening period, patients returned to their usual CU membrane.

The blood flow rate was maintained at 250-300 ml/min from an internal arteriovenous fistula and the dialyzate, which contained acetate, had a flow rate of 500 ml/min in a single pass mode. Heparin was administered at a dose between 2000 to 5000 U at the start and 250 to 1000 U hourly by continuous infusion.

Re-use of the dialyzers consisted of reverse ultrafiltration with tap water for 20 min and storage with 3 % formalin as described [23]. According to the manufacturers instruction, PAN was rinsed with 1 % hypochlorite prior continuing the re-use method.

Sample collection

Two ml whole blood were drawn from the patients arteriofistula before initiation of dialysis (time 0) and from the venous (outflow) line of the dialyzer at 5, 15, 60 min after the onset of dialysis. Blood was collected into tubes containing potassium EDTA (3,3 mg). The white blood cell counts were performed with an automated hematology analyzer (Sysmex CC-800) and neutrophil differential counts were carried out manually. Plasma was removed by centrifugation, stored at −80° C and thawed once for determination of complement.

Complement measurement

The plasma level of C_{3d} generated by complement activation was measured in a two step procedure [24]. Native C_3 and high molecular weight fragments C_{3b} and C_{3c} were precipitated with polyethylene glycol. The C_{3d}-containing supernatant was assayed by single, radial immunodiffusion (RID) with anti-C_{3d} serum (Behring Werke AG).

Statistical analysis

Wilcoxon signed rank test for matched pairs were used for statistical analysis unless otherwise stated. All the data are the mean ± SEM.

Results

Effect of re-use on neutrophil counts

As shown in figure 1, the 5 membranes induced a significant neutropenia during their first use. Neutropenia appeared most extensive on CU (6 ± 1 %), intermediate on PC and CA (45 % ± 11

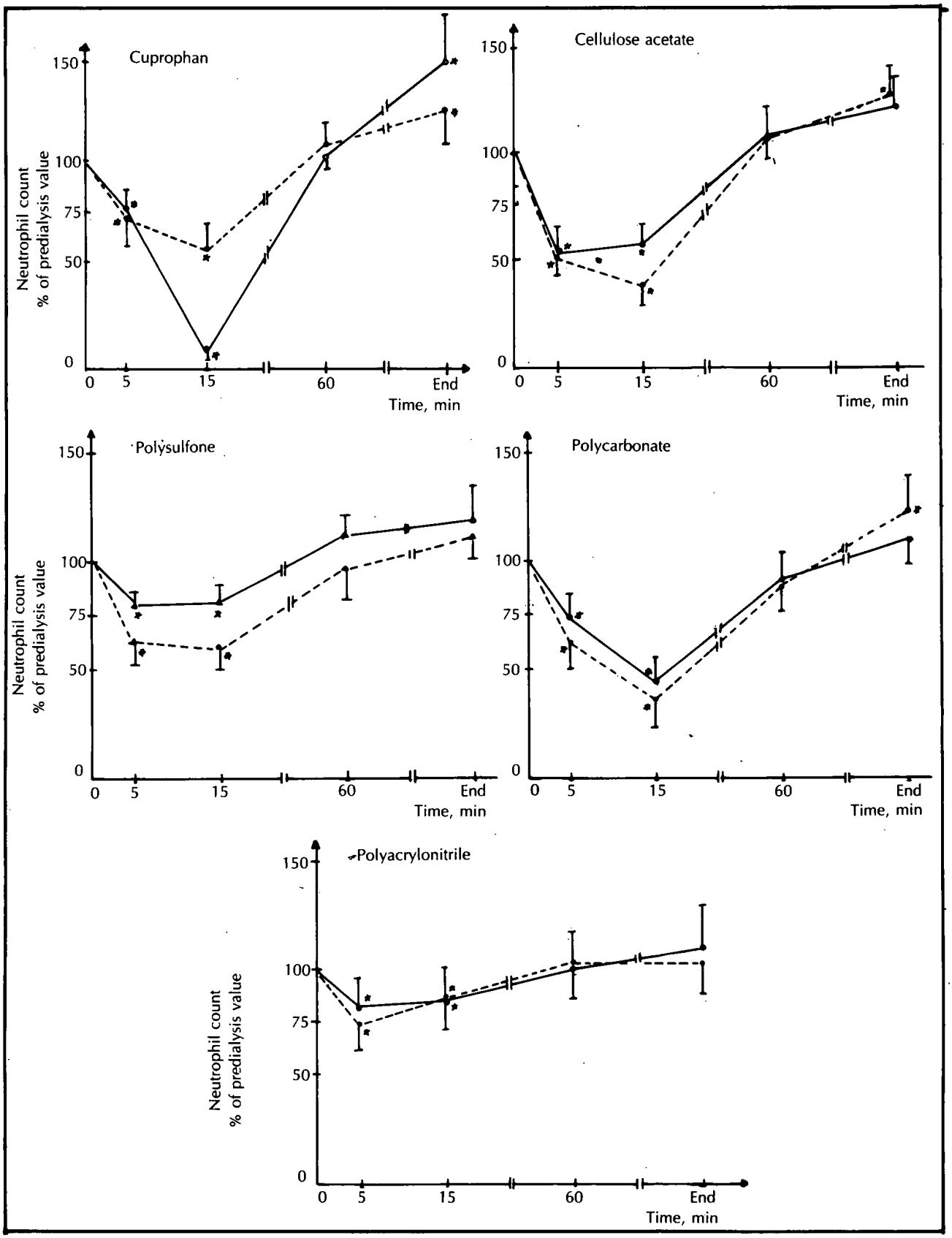

Fig. 1.—Time course of neutrophil count during first use (open symbols) and second use (brokenlines with closed symbols) of different dialysis membranes.

and 49 % ± 9 respectively) and least on PS and PAN (78 % ± 4 and 83 ± 12 respectively). Maximum neutropenia was observed as early as 5 min on CA, PS and PAN and at 15 min on CU and PC. Only, CU showed a significant rebound phenomenon at the end of dialysis.

Upon re-use neutropenia improved on CU only (66 % ± 12) $p < 0.05$. No significant improvement was observed with re-use of CA (36 % ± 8), PS (58 % ± 6), PC (32 % ± 23) or PAN (74 % ± 12). Rather a significant decrease of neutrophil count was observed on PAN compared to first use ($p < 0.05$). This could be however attributed to the different rinsing procedure applied to PAN (hypochlorite). At the end of dialysis, a significant rebound of neutrophil count was evidenced on CU, CA and PC. It can be observed that CA and PS switched the time of their maximum neutropenia from 5 to 15 min.

Effect of re-use on complement activation

The complement activating capacity of the different dialyzer membranes is shown in table I. At the maximum neutropenia, a significant generation of C_{3d} was observed except on PAN.

Re-use produced improvement of C_{3d} values at maximum neutropenia on CU only. However, by comparing the degree of neutropenia and the level of C_{3d} accumulated, striking differences can be observed between the membranes. A similar amount of C_{3d} appeared on CU and CA during first use, but the extent of the neutrophil fall in CU was almost twice as large as that observed on CA ($p < 0.001$, paired t-test). During first and second use of PC and PS, identical amounts of C_{3d} were generated, whereas the neutrophil drop on PC was twice that on PS ($p < 0.002$, paired t-test).

The maximal quantity of C_{3d} detected is shown in table II. The maximum C_{3d} generated coincided with the maximum neutropenia only on CU and CA first and second use and PS first use.

Discussion

Neutropenia and complement activation have been shown to represent useful parameters in evaluating the biocompatibility of dialyzer membranes. The present study shows that, on first use, CU as expected appears the least biocompatible, PS and PAN the most biocompatible, while CA and PC occupy an intermediate position.

The improved neutropenia together with complement activation as a result of re-use is well recognized on cuprophan membranes [19-23]. Our results confirm this observation. In contrast, CA, as shown by others [22], as well as PS and PC did not improve neutropenia and complement activation upon their second use. Moreover, a significant worsening of the slight neutrophil fall was observed on PAN during second use, while no significant sign of complement activation was evidenced. The enhanced biocompatibility of re-used CU has been shown, at least in part, to result from deposition of C_{3b} on the membrane surface, thus blocking complement activating sites [3]. The absence of improvement of neutropenia and complement activation on CA, PS, PC and PAN suggest that the protective mechanism described for CU cannot be applied to modified cellulosic and synthetic membranes. These results support the idea that either the plasma proteins and/or C_{3b} were not fixed by these membranes or that the reprocessing technique may modify the protein coating

Table I. Effect of re-use on C_{3d} generation and neutropenia induced by different dialyzers.

Dialyzer		C_{3d} concentration mg/100 ml At time 0	C_{3d} concentration mg/100 ml At maximum neutropenia	ΔC_{3d}	Neutrophil count % of predialysis values At maximum neutropenia
CU	I	1.49 ± 0.23	3.02 ± 0.21 *	1.53	6.2 ± 1.2 * (15)
	II	0.92 ± 0.18	1.73 ± 0.15 *	0.81	57.6 ± 14.8 * (15)
CA	I	1.78 ± 0.23	3.07 ± 0.31 *	1.23	52.7 ± 12.6 * (5)
	II	1.22 ± 0.28	2.58 ± 0.31 *	1.36	36.8 ± 9.3 (15)
PS	I	2.27 ± 0.21	2.83 ± 0.34 *	0.56	77.9 ± 4.2 * (5)
	II	1.49 ± 0.17	2.30 ± 0.26 *	0.81	57.6 ± 5.9 * (15)
PC	I	1.62 ± 0.26	2.18 ± 0.31 *	0.56	45.1 ± 11.1 * (15)
	II	1.79 ± 0.10	2.51 ± 0.26 *	0.72	31.5 ± 12.1 * (15)
PAN	I	1.58 ± 0.17	1.87 ± 0.17	0.29	83.0 ± 14.4 * (5)
	II	1.42 ± 0.22	1.67 ± 0.21	0.25	74.0 ± 12.3 * (5)

* = $p < 0.02$ versus predialysis values. Brackets] indicate the significance between first (I) and second (II) use at $p < 0.05$. Number in brackets indicate the time of maximum neutropenia in min.

Table II. Maximum complement activation capacity of the different dialyzers

		C_{3d} concentration mg/100 ml	Time of dialysis	ΔC_{3d}
CU	I	3.02 ± 0.21 *	15	1.53
	II	1.73 ± 0.15 *	15	0.81
CA	I	3.01 ± 0.31 *	5	1.23
	II	2.58 ± 0.31 *	15	1.36
PS	I	2.83 ± 0.34 *	5	0.56
	II	2.56 ± 0.18 *	60	1.07
PC	I	2.30 ± 0.36 *	end	0.68
	II	3.21 ± 0.30 *	end	1.42
PAN	I	2.03 ± 0.21 *	15	0.45
	II	1.78 ± 0.38	end	0.36

* = $p < 0.05$ versus predialysis values.

differently according to the nature of the membrane. In addition, the neutrophil drop observed upon re-use of PAN could also be explained by the use of hypochlorite rinsing, which has been shown to remove the protective protein coating [21]. The biocompatibility of the 5 re-used membranes according to the present reprocessing technique was thus modified as to find CU at the level of PS and PAN, while CA and PC kept their intermediate position.

The development of a radioimmunoassay for C_{3a} has added further support to the hypothesis that complement activation is the cause of dialysis leukopenia [25]. It was found that new CU activated eight to ten times more complement than re-used CU, two times more than new CA and 20 times more than new PAN. The degree of hemodialysis-associated leukopenia produced by these dialyzers was inversely related to their complement activating capacity. More recently, assessment of the generation of the complement fragment C_{3d} was shown to be an appropriate cumulative marker of complement activation during hemodialysis [14, 15]. Regenerated cellulose was found to cause significantly more leukopenia and generation of C_{5a} than cellulose acetate in association with the greatest increase in plasma C_{3d} [14]. Using CU dialyzers, the same kinetic pattern was observed for the generation of C_{3a}, C_{5a} and C_{3d} [15].

Our results show that complement activation was linked to neutropenia, however the magnitude of activation did not completely correlate with the degree of neutropenia. Indeed, during the use of new CU and CA, almost identical amounts of C_{3d} were generated despite large differences in neutropenia. The same was observed between PS and PC. Since C_{3bi}, which contains the C_{3d} epitope has been shown to be membrane-bound on CU [10] measurement of C_{3d} might underestimate complement activation on this membrane. PAN was also found to adsorb C_{3a} desarg and C_{5a} desarg [3, 9]. However, this adsorption phenomenon has not been demonstrated for PS or PC and thus cannot explain the discrepancies observed between these two synthetic membranes.

In summary, the results presented demonstrate that re-use of dialyzers improves the biocompatibility of CU only, but not of modified cellulose or synthetic membranes. C_{3d} determination appears a stable and sensitive index of complement activation. Finally the correlation between complement activation and neutropenia was not firmly established on all the membranes tested.

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