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Biocompatibility studies on cellulose and synthetic haemodialysis membranes

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ESTUDIOS DE BIOCOMPATIBILIDAD CON MEMBRANAS DE CELULOSA Y SINTETICAS

RESUMEN

El objetivo de este trabajo fue estudiar y comparar los cambios producidos en los parámetros de bio(in)compatibilidad, como son las modificaciones en el recuento de neutrófilos, activación del complemento y las variaciones gasométricas de los distintos tipos de dializadores durante la hemodiálisis (HD). Los pacientes fueron tratados con las siguientes membranas: cuprofán, poliacrilonitrilo (capilar y placa), gambrane y polisulfona. Durante la HD con cuprofán los granulocitos descendieron de forma muy significativa hasta el 25 % del valor inicial a los quince minutos de la diálisis. A ese mismo tiempo la neutropenia con el gambrane fue del 60 %, con el poliacrilonitrilo del 84 % y con la polisulfona del 95 % con respecto a las cifras basales. Existían diferencias muy significativas entre el cuprofán y el resto de las membranas. No se observaron variaciones entre poliacrilonitrilo en sus dos formas y polisulfona. Sin embargo, sí había diferencias entre poliacrilonitrilo y polisulfona con relación al gambrane ($p < 0,05$). No se encontraron modificaciones importantes entre los diferentes tipos de membranas en lo que respecta a la activación del complemento, valorado mediante la determinación del CH50. Los cambios observados en las cifras de PaO_2 y $PaCO_2$ fueron comparables en todos los dializadores. La repercusión clínica a largo plazo de estos fenómenos no se puede valorar con los resultados obtenidos en este estudio. Es probable que las variaciones en la inducción de leucopenia y activación del complemento entre cuprofán y las otras membranas sean debidas a las diferencias en la estructura de su superficie y su composición química. En un futuro, las modificaciones de estos parámetros en la membrana de cuprofán pueden producir resultados comparables a los obtenidos con otros tipos de membranas sintéticas.

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

SUMMARY

The aim of this report has been to study and compare the widely used indicators of bio(in)compatibility, namely white blood cells (WBC), activation of complement and gas changes on patients treated with regular hemodialysis. The patients underwent dialysis with different kinds of membranes: cuprophane, polyacrylonitrile (hollow fiber and flat sheet), gambrane and polysulfone. In subjects using cuprophane the WBC decreased to 28 % of predialysis values at 15 minutes.

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At the same time the equivalent values for gambrane were 60 %, polyacrylonitrile 84 % and polysulfone 85 %. These differences reached a statistical significance in all cases when they were compared to cuprophane. No differences were observed between polyacrylonitrile and polysulfone. But the values obtained for these membranes were significant in relationship with gambrane ($p < 0.05$). Complement activation as measured by CH50 levels failed to distinguish among cuprophane and sinthetic membranes. PaO_2 and PCO_2 changes were comparable for all dialyzers. The long term clinical significances of these phenomena remains unanswered and further studies are needed. Since it is likely that the differences in leukopenia and complement activation between cuprophane and sinthetic membranes are due to differences in the dialyzer surface and interaction with blood, alterations in membrane chemistry for cellullose based membrane such as cuprophane may eventually yield results comparable with those for sinthetic membranes.

Key words: ***Biocompatibility. Complement. Hemodialysis. Leucopenia. Membranes.***

Introduction

Haemodialysis is a well established and widely used mode of treatment for both acute and chronic renal failure. The technique of treatment relies upon the diffusion of metabolites elevated as a consequence of renal insufficiency across a semipermeable membrane contained in the haemodialyser into a fast flowing electrolyte solution (dialysis fluid).

The most commonly used membrane is Cuprophan ® (Enka Ag, Wuppertal-Barmen, FRG), a cellulose based membrane manufactured by the cuprammonium process. A number of variants of this technique of manufacture exist leading to modified cellulose membranes such as cellulate, Saponified Cellulose Ester (SCE), (CD Medical, Florida, USA). The past decade has also seen the development and clinical application of synthetic co-polymer membranes such as polyacrylonitrile (AN-69) (Hospal, Lyons, France); PAN 15 (Asahi Medical, Tokyo, Japan); polysulfone (Fresenius Ag, Bad Homburg, FRG), and polycarbonate (Gambrane ®) (Gambro Ab, Lund, Sweden).

The benefits of renal replacement therapy has long been recognised. It was known as early as 1968 that treatment is associated with a transient and rapidly reversible fall in white cell count [1]. This fall and subsequent return to predialysis levels is due to a fall in neutrophils, monolytes and eosinophils which occur over the same time span as the leucopenia [2]. Acceptance of this phenomenon was due to the fact that it was without symptoms and sequelae [1] and there was little opportunity in studying the phenomenon since at that time all clinically used membranes were manufactured by the cuprammonium process.

In 1977 Craddock and colleagues published a series of papers describing a possible mechanism for leucopenia and linking it with hypoxia and complement activation [3, 4]. Although this elegant hypothesis was not universally accepted [5, 7], the availability of the newer haemodialysis membranes resulted in extensive study of the bio(in)compatibility of haemodialysis membranes.

A number of indicators of bio(in)compatibility have been described. These include the white cell changes [8], activation of complement [9], leucocyte function [10], disorders of blood coagulation and fibrinolysis [11], granulocyte adherance [12], whole blood chemiluminescence [13] as well as release of leucocyte elastase [14]. The aim of our clinical investigation has been to study and compare the widely used indicators of bio(in)compatibility, namely white cell changes, activation of complement and to perform blood and expired gas measurements on patients receiving dialysis treatment using cellulose based and synthetic haemodialysis membranes.

Materials and methods

a) *Membranes*

Details of the membranes studied are shown in Table I.

b) *Patients and Dialysis Procedures*

All patients studied were receiving regular dialysis treatment for chronic renal failure. All studies were performed after informed consent and Ethical Committee approval received.

None of the patients studied had signs of pulmonary insufficiency, congestive heart failure nor were they taking medications known to affect white cell count. Smoking was not permitted throughout dialysis.

Vascular access was by arterio-venous fistulae using two needles with a blood flow being maintained at

Table I. Membrane Types Studied

Group	Membrane	Format	Surface area of device incorporating membrane (m^2)	Membrane manufacturer
Cellulose	Cuprophan	Hollow fibre	1.3	Enka Ag, Wuppertal-Barmen, FRG
Synthetic	Polyacrylonitrile	Hollow fibre	1.0	Hospal, Lyons, France
		Flat sheet	1.15	Hospal, Lyons, France
	Gambrane	Flat sheet	1.0	Gambro Ab, Lund, Sweden
	Polysulfone	Hollow fibre	0.7	Fresenius Ag, Bad Homburg, FRG

200 ml/min during the studies. Heparin was used as the anticoagulant. Treatment duration was four hours and two different types of proportionating systems were used (Lucas 11, Fresenius Dylade E). They differed only in their ability to control ultrafiltration. Acetate based dialysis fluid (40 mmol/l) was used in all studies. The number of patients using each of the membrane types studied ranged from five to eight.

c) *White cell changes and complement activation*

Pre dialysis blood samples were obtained from the arterial cannula at the time of insertion and from the arterial portion of the extracorporeal circuit at 5, 10, 15, 20, 30, 45, 60 and 120 minutes during dialysis. White cell counts were determined by the use of a Coulter counter. Complement activation was monitored by CH50 levels which were measured using standard techniques, split products (C3a and C3d) levels were assayed using a commercially available radioimmunoassay kit (Upjohn Diagnostics, Kalamazoo, Michigan, USA) and an immunochemical assay in conjunction with a Roche Cobas Biocentrifugal analyser. Samples for complement levels were taken pre dialysis and at 15, 60 and 120 minutes during treatment. They were placed in K_2 EDTA tubes, separated within 30 minutes and stored in aliquots at −70° C until assay.

d) *Blood and expired gas measurements*

Blood gas measurements were performed on 1 ml samples drawn from the arterial and venous segments of the extracorporeal circuit.

Samples were drawn over a 30 second period into syringes whose dead space was filled with heparin (approx 200 IU). Sampling times were 15, 60 and 120 minutes during treatment as well as pre dialysis. The samples were placed on ice immediately and analysed by an ILS 1302 or Corning 168 blood gas analyser within thirty minutes of sampling.

Carbon monoxide diffusing capacity or transfer factor (D_LCO) was measured predialysis and at 15, 60 and 120 minutes during treatment. Measurements were performed with patients in an upright position using a Morgan transfer factor machine (PK Morgan, Chatham, UK). Details of the technique of measurement are given in Cotes [15].

Results

All results have been expressed as a percentage of predialysis value and the mean ± standard deviation calculated. Mean results obtained for synthetic membranes were compared with those obtained for Cuprophan using a student's t test. A value of $p < 0.05$ was considered to be significant.

a) *White cell counts*

In patients using Cuprophan membranes the white cell count had fallen to 28.6 ± 8.2 % of predialysis levels by 15 minutes. In contrast, equivalent values for Gambrane were 60.8 ± 16.9 %, 84.5 ± 7.3 % for polyacrylonitrile and 85.7 ± 9.0 % for polysulfone, the differences reaching a statistical significance compared to Cuprophan. Although we were unable to distinguish between polyacrylonitrile and polysulfone at this time, the values observed for these two membranes differed from that for Gambrane ($p < 0.05$). By sixty minutes, white cell counts had returned to predialysis levels for all membranes.

b) *Complement activity*

Results obtained for CH50 levels are summarised in Table II. Small but statistically non-significant changes in values during dialysis were noted during dialysis. An inter membrane comparison yielded no differences between Cuprophan and the synthetic membranes.

C3a and C3d changes observed are summarised in Table III. There are marked differences between the two groups of membranes in the case of C3a and for polyacrylonitrile, Gambrane and polysulfone membranes considerable standard deviations were observed. The reason for the wide variation observed is unclear, but there are two principal possibilities: the first is patient response; the second assay variability or non specificity. This aspect of membrane biocompatibility is the subject of continuing study.

Table II. Total Haemolytic Complement Levels (CH50)

Membrane	Format	Time (min)		
		15	60	240
Cuprophan	HF	83.8 ± 18.3 (6)	94.1 ± 13.9 (6)	104.9 ± 15.1 (5)
Polyacrylonitrile	FS	93.9 ± 9.5 (6)	100.9 ± 5.5 (6)	94.8 ± 7.8 (4)
Gambrane	FS	87.1 ± 9.6 (6)	96.8 ± 5.5 (6)	—

All data presented as percentage of pre dialysis values expressed as Mean ± SD.
Figures in parentheses refer to number of observations.

Table III. Venous C3a and C3d levels during haemodialysis

Membrane	Format		Time (minutes)			
			15	60	120	240
Cuprophan [1]	HF	C3a	539.6 ± 581.6 (6)	258.9 ± 56.6 (6)		139 ± 46 (6)
		C3d	116.7 ± 24.8 (6)	113.4 ± 3.5 (6)		142.6 ± 22.4 (6)
Polyacrylonitrile	FS	C3a	102.4 ± 20.5 (6)	84.7 ± 26 (6)		73.6 ± 32.1 (6)
		C3d	99.9 ± 27.2 (6)	109.2 ± 34.9 (6)		86.4 ± 26.2 (6)
	HF	C3a	509.8 ± 461 (4)	1023.9 ± 1824.8 (6)		
		C3d	122.6 ± 15.4 (12)	117.2 ± 22.1 (12)	118.5 ± 20.5 (12)	
Gambrane	FS	C3a	1919 ± 1517 (4)	610 ± 532 (5)	419 ± 279 (4)	
		C3d	117.1 ± 32.8 (10)	107.6 ± 22 (10)	114.2 ± 23.3 (10)	
Polysulfone	HF	C3a	1089 ± 990 (4)	329 ± 328 (5)	3018 ± 5245 (5)	
		C3d	106 ± 14 (10)	107.5 ± 17.3 (10)	113.6 ± 18.1 (10)	

[1] Measurements on arterial samples.
All data presented as percentage of the pre dialysis value expressed as Mean ± SD. Values in parentheses refer to number of measurements.

Due to the wide standard deviations noted, no statistical comparison of the membranes was undertaken. C3d levels remained comparable throughout dialysis and no inter membrane differences were noted.

c) *Blood gas changes*

Arterial oxygen (PaO_2) changes are shown in Table IV. All membranes showed a fall in this parameter at 15 minutes compared to predialysis levels. Synthetic membranes were similar in the falls they produced, although in the case of polysulfone it was similar. Levels remained below predialysis levels throughout dialysis. Arterial carbon dioxide levels ($PaCO_2$) also fell during dialysis but our results failed to differentiate between Cuprophan and synthetic membranes.

d) *Transfer factor*

The patient's carbon monoxide diffusing capacity during dialysis is shown in Table V. A fall with each of the membranes was noted. The greatest fall was for Cuprophan (70.2 ± 13.9 % of predialysis levels at 15 minutes) compared with 90.5 ± 15 % for Gambrane,

Table IV. Blood gas (PaO_2, $PaCO_2$) changes during haemodialysis

Membrane	Format		Pre dialysis levels (kpa)	Time (minutes) 15	60	120
Cuprophan [1]	HF	PaO_2	12.6 ± 1.4	87.7 ± 12.4 (8)	83 ± 9.9 (8)	85.3 ± 11.6 (8)
		$PaCO_2$	45 ± 0.5	92.2 ± 3.1 (8)	97.7 ± 3.5 (8)	97.1 ± 3.4 (8)
Polyacrylonitrile	FS	PaO_2	15.5 ± 2.0	98 ± 9.5 (6)	78.3 ± 9.9 (6)	75.6 ± 3.5 (6)
		$PaCO_2$	48 ± 0.7	97 ± 4.0 (6)	98.3 ± 6.0 (6)	96.3 ± 7.2 (6)
Gambrane	FS	PaO_2	14.8 ± 1.7	96.2 ± 14.2 (5)	90.9 ± 27.9 (5)	80 ± 7.5 (5)
		$PaCO_2$	4.8 ± 0.5	96.9 ± 3.2 (5)	96.9 ± 10.2 (5)	98.5 ± 4.2 (5)
Polysulfone	HF	PaO_2	15.6 ± 2.5	82.8 ± 19.8 (5)	80.4 ± 14.5 (5)	73.8 ± 5.7 (5)
		$PaCO_2$	5.4 ± 0.5	98.7 ± 6.6 (5)	93.3 ± 5.0 (5)	89.2 ± 4.9 (5)

All data presented as percentage of the pre dialysis value expressed as Mean ± SD. Values in parentheses refer to number of measurements.

Table V. Transfer factor (D_LCO)

Membrane	Format	Pre dialysis levels (*)	Time (mins) 15	60	120
Cuprophan	HF	a	70.2 ± 13.9 (8)	61.3 ± 12.16' (8)	74.6 ± 13.5 (8)
Polyacrylonitrile	HF	5.88 ± 1.81	94.2 ± 8.7 (5)	88.5 ± 6.9 (5)	89.6 ± 4.6 (5)
Gambrane	FS	6.88 ± 0.87	90.5 ± 15.0 (5)	77 ± 11.4 (5)	85.2 ± 10.2 (5)
Polysulfone	HF	7.02 ± 1.02	86.4 ± 9.6 (5)	85.2 ± 4.7 (5)	82.1 ± 6.8 (5)

All data presented as percentage of pre-dialysis values expressed as Mean ± SD. Figures in parentheses refer to number of observations.
a = data unavailable.
* = mmol/min/kpa.

94.2 ± 8.2 for polyacrylonitrile and 86.4 ± 9.6 for polysulfone. For all membranes the values remained below predialysis at 120 minutes. An intermembrane comparison demonstrated no significant differences between synthetic membranes at all the sampling times. When the values for Cuprophan were compared with synthetic membranes the differences were significant ($p < 0.05$) at 15 and 60 minutes.

Discussion

Our results for white cell changes confirm published data from other groups [14, 16, 17]. Complement activation as measured by CH50 levels failed to differentiate between synthetic and cellulose based membranes.

C3a levels differed between synthetic membranes and that based on cellulose. A wide variability in the results was noted and this is under further study to try and elucidate the reason. It is well recognised that there are significant differences between synthetic and cellulose based membranes in this measurement, a likely explanation confirmed by the studies of Cheung [18] is that differences between the surface layering and adsorption characteristics of the membranes exist and this results in the binding of C3a to the membrane surface.

C3d levels also rose during dialysis but the changes noted failed to reach statistical significance.

Hypoxia during haemodialysis is well recognised. A number of postulates as to its cause have been proposed and these have been reviewed in the literature. Our results show that the most severe fall in PaO_2 is with Cuprophan. Rather surprisingly we have observed a marked similarity in this parameter for all membranes studied, a result that is at variance with those reported from our group in an earlier study [19]. $PaCO_2$ changes were comparable for all membranes.

Changes in D_LCO on the other hand, mirror changes in the white cell count. The time course of these changes differs in as much as leucopenia is reversed by the end of the first hour, while D_LCO remains depressed throughout dialysis. This depression may be a consequence of acute lung damage during the leukostasis.

Although the differences between membranes in respect of the parameters studied assume a statistical significance, their clinical implications are poorly understood. Evidence has been presented in the literature linking «first use syndrome» with activation of complement [20]. Furthermore, it is recognised that the hypoxia observed during dialysis may be ameliorated or eliminated by the use of bicarbonate based dialysate [6, 7]. The study of lung function of patients has received little attention although Lee [21] and Wolf [22] have demonstrated restrictive ventilatory changes possibly due to fibrotic changes as well as a more generalised decrease in carbon monoxide diffusing capacity in addition to the acute changes of our own study. As to whether these changes are a consequence of renal failure or associated anaemia or fluid overload, or possibly a consequence of repeated exposure to activated neutrophils, remains as yet unanswered, but since cardio-pulmonary complications remain the most frequent cause of death in patients receiving regular dialysis treatment, further studies are clearly needed to answer this important question.

Conclusions

Our studies have confirmed those of other groups who have studied white cell changes and complement activation and demonstrate less bio(in)compatibility when synthetic membranes are used. The long term clinical significance of these phenomena remains unanswered and further studies to establish their long term implications are needed, particularly as at present there in a considerable price differential between cellulose based and synthetic membranes. Since it is likely that the differences in leucopenia and complement activation between Cuprophan and synthetic membranes are due to the differences in the membrane surfaces and their interaction with blood, alterations in membrane chemistry for cellulose based membranes such as Cuprophan may eventually yield results comparable with those for synthetic membranes. This approach has indeed been adopted by the manufacturers of cellulose haemodialysis membranes and first published data confirm reduction in both leucopenia and complement activation with such membranes [23, 24].

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