JOURNAL OF MATERIALS SCIENCE MATERIALS IN MEDICINE 4 (1993) 192–195

Poly(butylene terephthalate) haemocompatibility after physicochemical treatment in order to increase wettability

E. CENNI, L. SAVARINO, S. STEA, D. CAVEDAGNA, M. E. DONATI, G. MARI*, G. FALSONE‡, A. PIZZOFERRATO
*Laboratory for Biocompatibility Research on Implant Materials, Istituti Ortopedici Rizzoli, Bologna, Italy *Biofil SRL, Cavezzo, Modena, Italy‡ Centro Regionale per il Servizio Trasfusionale, USL 27, Bologna, Italy*

We evaluated the haemocompatibility of a filter of poly(butylene terephthalate) for packed red cell transfusions, which had undergone a physicochemical treatment (corona treatment) in order to reduce the filling time. Leucocyte, erythrocyte and platelet counts were carried out and haematological and coagulation parameters were examined before and after filtration. Moreover, some biochemical plasma parameters were evaluated, which are normally checked in hospitalized patients. The results obtained show that the filter treatment reduces the filling time, does not reduce the efficiency in retaining leucocytes and platelets, and does not induce alterations in filtered red blood cells. However, the treatment prolongs the activated partial thromboplastin time (APTT) and reduces the activity of some factors of the intrinsic coagulation pathway. On the other hand, it does not induce modifications in the biochemical parameters examined.

1. Introduction

Most non-haemolytic reactions after transfusions of packed red cells are caused by the presence of leucocytes and platelets. In patients receiving transfusions, leucocytes and platelets can induce hypersensitivity. In order to avoid such adverse reactions, the transfusion of packed red cells should occur by using special filters capable of retaining leucocytes and platelets and which let red blood cells through without altering them [1–3]. These filters, previously made of cellulose acetate or cotton wool, are now manufactured from polyester. Filling the filter with blood and consequently eliminating air in a packed red cell unit through a common polyester filter is a long procedure that takes between 15 and 45 min, depending on the blood haematocrit, temperature and age. That is very time-demanding for the nursing staff.

In order to shorten the filling time for packed red cells, the wettability of a polyester filter was enhanced with a physical surface treatment. Some investigations were carried out to verify whether this treatment altered the efficiency in retaining leucocytes and platelets and could cause alterations in filtered blood red cells. Also, modifications of coagulative and biochemical parameters of the blood to be transfused were evaluated.

2. Materials and methods

2.1. Materials

Two types of filters for haemotransfusion were analysed, manufactured by Biofil SRL (Cavezzo, Modena, Italy). Filter 1 was made of 19 layers of poly(butylene terephthalate), held by two layers of broader-weft polyester fabric. Poly(butylene terephthalate), a linear-chain hydrophobic polymer obtained with the "melt-blown" technique, is available as woven or non-woven fabric, with fibres of diameter 2–3 μm. Filter 2 was made of the same material modified through corona treatment at atmospheric pressure in an air flow. This treatment was performed by means of a 6 kW r.f. corona discharge apparatus with knife-type electrodes, coupled with a 50–15 000 Hz frequency converter. The current intensity during the discharge was 2 A. The treatment was carried out continuously, starting from 40 cm-wide non-woven spools, on both the material surfaces.

The feed speed of the material was 3 m min^{-1} with the electrodes at a distance of 2 mm from the material under treatment. The treatment effect was quantified by determining the height of the water column required to wet the material. The water column height was increased at a rate of 1.3 cm s^{-1}.

Before treatment the material required a column height of 28 cm to become wet. This height was reduced to 5 cm after treatment. At the end of the treatment the spools were cut and assembled into their final shape.

Corona treatment induced the formation of polar groups that cause an increase both in the wettability and in the adhesion forces of the polar compounds. The increase in polarity derives both from oxidation

0957–4530

phenomena and from the formation of double bonds at the surface, in particular CO groups. The presence of these groups is limited to a very thin surface layer, with a thickness of about 100 nm. In both filters each layer was 1 mm thick, but the total thickness of the filter was 11 mm because the layers were pressed in a frame.

2.2. Methods

Two criteria were followed in choosing the tests: to evaluate whether the filter could retain platelets and leucocytes without altering the red blood cells, and whether it had adverse effects on coagulation factors, enzymes, electrolytes and other components of the blood to be transfused.

The studies were performed on 5 units of fresh blood collected in CPD-A (sodium citrate 2.63 g, citric acid 0.30 g, biphosphate-sodium 0.22 g, destrosium 2.55 g and adenine 27.5 mg), examined within 3 h after collection. One-third of the blood contained in each bag was filtered through filter 1, one-third was filtered through filter 2 and one-third was not filtered at all. On the two fractions of filtered blood and, by comparison, the unfiltered fraction, the following tests were performed.

1. Counting of erythrocytes (RBC), leucocytes (WBC), platelets (PLT), leucocytic differential counting and determination of haemoglobin (Hgb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW) by a Coulter Counter JS instrument.
2. Determination of the osmotic erythrocytic fragility (haemolysis).
3. Determination of PT, APTT and clotting factors (XII, XI, IX, VIII, VII, X, V, II and fibrinogen).
4. Evaluation of inhibitors of blood coagulation: assay of antithrombin III and C-protein by chromogenic methods.
5. Evaluation of fibrinolysis: assay of plasminogen and α_2-antiplasmin by chromogenic methods, assay of fibrin degradation products (FbDP) with latex agglutination test and assay of fibrinogen degradation products (FgDP) with enzyme immunoassay.
6. Evaluation of biochemical parameters: assay of urea, creatinine, total proteins, cholesterol, triglycerides, calcium, phosphorus, total bilirubin, uric acid, AST, ALT, γ-GT, alkaline phosphatase, LDH, α-HBDH, pseudocholinesterase, CK, amylase, magnesium by spectrophotometric methods and assay of plasma potassium by flame photometry.
7. Electrophoresis of plasma proteins.
8. Assay of immunoglobulins G, A and M and of the C3 and C4 complement subunits by nephelometer.
9. Counting of blood cells in the saline wash solution of each filter layer. To carry out the count, the filter was opened and each layer was soaked in 50 ml saline solution for 30 min. In the wash solution red blood cells, leucocytes and platelets were counted.

TABLE I Mean values ± SD (five experiments) of erythrocyte, leucocyte and platelet counts before and after blood passage through the filters examined

Test[a] (unit)	Unfiltered blood	Filtered blood	
		Filter 1	Filter 2
RBC ($\times 10^6$ µl^{-1})	4.20 ± 0.19	4.04 ± 0.36	4.04 ± 0 36
Hgb (g dl^{-1})	12.5 ± 0.70	11.7 ± 0.84	11.8 ± 0.78
Hct (%)	37.9 ± 2.34	36.5 ± 2.06	36.5 ± 2 07
MCV (fl)	90.2 ± 4.48	90.6 ± 5.06	90.6 ± 5.04
MCHC (g dl^{-1})	32.7 ± 1.12	32.1 ± 1.03	32.4 ± 0.78
RDW (%)	13 3 ± 0.90	13.1 ± 0.79	12.8 ± 0 80
WBC ($\times 10^3$ µl^{-1})	7.1 ± 1.96	0 1 ± 0.11	0.2 ± 0 16
PMN ($\times 10^3$ µl^{-1})	4.3 ± 1.67	Unreliable	Unreliable
Lymph ($\times 10^3$ µl^{-1})	2.9 ± 0 48	Unreliable	Unreliable
Mono ($\times 10^3$ µl^{-1})	0.2 ± 0.1	Unreliable	Unreliable
PLT ($\times 10^3$ µl^{-1})	238 ± 19.86	3 ± 2.62	2 ± 0.97

[a] RBC, red blood cells, Hgb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; WBC, white blood cells; PMN, polymorphonuclear granulocytes, Lymph, lymphocytes; Mono, monocytes; PLT, platelets.

3. Results

The filtering time of a bag of fresh whole blood through filter 1 was 57 ± 28.9 s (mean ± SD, five experiments), and the filtering time through filter 2 was 21 ± 7.94 s.

The results of the tests performed before and after the filtering of fresh whole blood are summarized in the following tables. In Table I the results of blood cell counting are listed. Only minor variations of red cells were reported after filtration through either filter 1 or filter 2. The retention percentage was 3.8% in both filters, haemoglobin was decreased by 6.4% in filter 1 and by 5.6% in filter 2, haematocrit was decreased by 3.7% and MCV was increased by 0.4% in both filters, MCHC was reduced by 1.8% in filter 1 and by 0.9% in filter 2. These alterations were not significant concerning the transfused red cell quality.

The filters showed a similar efficiency in retaining leucocytes and platelets. Leucocyte retention was 98.6% in filter 1 and 97.2% in filter 2. Platelet retention was 98.7% in filter 1 and 99.2% in filter 2.

It is not possible to report the results of the differential counting of leucocytes in blood after filtration, because their number was too small to allow a reliable counting.

Neither of the filters produced variations in osmotic erythrocytic fragility, which is determined by diluting red blood cells with progressively increasing amounts of NaCl. Fig.1 shows the osmotic fragility curve of red blood cells in unfiltered blood, in the blood filtered through filter 1 and in that filtered through filter 2. It can be noted that the three curves are almost identical.

In Tables II and III the results of coagulation tests are listed. Filter 1 did not present relevant variations with respect to unfiltered blood. The most remarkable variation in filter 2 was an increase in APTT by 22.6%, which was confirmed by a strong reduction in factor XI activity (76.5%). No relevant variation in inhibitors of coagulation factors or in fibrinolysis was observed.

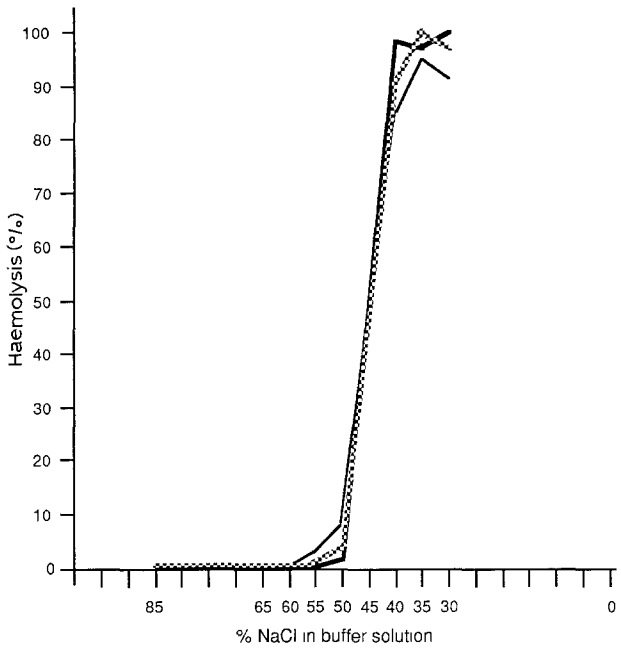

Figure 1 Osmotic erythrocyte fragility (——) before and after filtration through (——) filter 1 and (○ ○ ○) filter 2.

TABLE II Coagulative parameters before and after blood passage through the filters examined

Test (unit)	Unfiltered plasma	Filtered plasma	
		Filter 1	Filter 2
APTT (s)	32.7	32.8	40.1
PT (s)	10.6	10.8	10.7
Factor XII (%)	80	80	80
Factor XI (%)	85	85	20
Factor IX (%)	150	100	150
Factor VIII (%)	100	95	90
Factor VII (%)	100	90	90
Factor X (%)	120	120	120
Factor V (%)	150	120	120
Factor II (%)	100	100	100
Fibrinogen (mg %)	218	222	258

TABLE III Values of some coagulation inhibitors and some fibrinolytic parameters, before and after blood passage through the filters examined

Test (unit)	Unfiltered plasma	Filtered plasma	
		Filter 1	Filter 2
Antithrombin III (%)	106	102	108
C-protein (%)	97	101	92
Plasminogen (%)	83	91	93
Antiplasmin (%)	98	100	97
FbDP (ng ml^{-1})	< 50	< 50	< 50
FgDP (ng ml^{-1})	16	14	17

In Table IV the mean ± SD values of some biochemical parameters of filtered blood that are usually assayed in patients are reported. Neither of the filters produced changes in these parameters.

Table V lists the values of protein fractions. Neither of the filters gave relevant modifications in the concentration of plasma proteins.

Table VI shows that neither immunoglobulins G, A and M nor C3 and C4 subunits underwent relevant variations after blood filtration through either filter.

The examination of the wash solutions of the different filter layers showed that red blood cells were retained in small amounts by both filters and in a similar way in every layer. Almost all leucocytes were retained before the ninth layer in filter 1 and before the tenth layer in filter 2. Platelets were retained mostly before the fourth layer in both filters. The leucocytes differential counting in the various filter layers showed that the different leucocyte populations were retained with the same modalities by the two filters. Lymphocytes and monocytes were mostly retained in the first layers, and granulocytes were usually retained later. However, with the leucocyte differential counting, as well as with the counting in wash solution, no white blood cells could be observed in either filter after the tenth layer, and this may indicate their efficiency in retaining leucocytes.

4. Discussion and conclusions

Although the values of the reported filtration times are referred to whole blood, it can be easily inferred that filter 2 has a filtration time less than half that of filter 1 also in the case of packed red cell units. Such a reduction in filtration time can solve nursing problems usually occurring when using common filters. Moreover, the increase in the wettability and consequently in the filtering speed determines a more homogeneous filling of the filter, a more efficient air elimination and a better use of the surface in order to remove leucocytes.

Filters 1 and 2 showed a similar efficiency in retaining leucocytes and platelets. Leucocyte retention was 98.6% for filter 1 and 97.2% for filter 2. Platelet retention was 98.7% for filter 1 and 99.2% for filter 2. Leucocytes are almost totally retained in the ninth or tenth layer, both in the untreated and in the treated filter. Platelets are completely retained in the first layers.

Both filters induced insignificant variations in red blood cells concerning transfusion, and did not increase their osmotic fragility.

The commercial filter did not induce any modification in the coagulation pathway. On the other hand, filter 2 prolonged APTT and reduced factor XI activity. However, these alterations are not relevant in transfusions of packed red cells, because the amount of plasma present in these units is very small.

Not only has the behaviour of blood cells and coagulation factors demonstrated the haemocompatibility of the filters examined, but the results of other biochemical parameters have also proven that these filters do not induce any alteration in the biochemical plasma balance.

In conclusion, the two filters have a similar efficiency in retaining leucocytes and platelets. They do not alter the number, the volume or the osmotic fragility of red blood cells. They do not modify the electrolytic or protein composition of the blood to be transfused,

TABLE IV Mean values ± SD of some biochemical parameters before and after blood passage through the filters examined

Test (unit)	Unfiltered plasma	Filtered plasma	
		Filter 1	Filter 2
Urea (mg %)	30 ± 6 7	29 ± 6.5	29 ± 6.9
Creatinine (mg %)	1.0 ± 0.1	1 0 ± 0.1	0.9 ± 0.1
Total proteins (g %)	5.9 ± 0.22	5.9 ± 0.22	5.8 ± 0.23
Cholesterol (mg %)	159 ± 20 9	160 ± 22.4	159 ± 24 1
Triglycerides (mg %)	64 ± 23.2	65 ± 24.6	65 ± 24.3
Calcium ($mEq\,l^{-1}$)	3.2 ± 0.24	3.1 ± 0.39	3.2 ± 0.29
Phosphorus ($mEq\,l^{-1}$)	7.2 ± 0 47	7.3 ± 0.52	7.0 ± 0.42
Total bilirubin (mg %)	0.5 ± 0.29	0.5 ± 0 28	0.5 ± 0.29
Uric acid (mg %)	3.8 ± 0.61	3.8 ± 0.58	3.7 ± 0.59
Magnesium ($mEq\,l^{-1}$)	1.02 ± 0.17	1.09 ± 0 16	1.31 ± 0 11
Plasmatic K^+ ($mEq\,l^{-1}$)	3.4 ± 0.22	3.4 ± 0.22	3.4 ± 0 14
AST ($U\,l^{-1}$)	7 ± 1.2	7 ± 1.3	7 ± 0.5
ALT ($U\,l^{-1}$)	7 ± 1.6	6 ± 0.9	6 ± 1.6
γ-GT ($U\,l^{-1}$)	9 ± 3.1	7 ± 2 5	8 ± 4.2
Alk. phosphatase ($U\,l^{-1}$)	71 ± 23.6	72 ± 23.4	74 ± 20 1
LDH ($U\,l^{-1}$)	110 ± 13.2	115 ± 17.4	112 ± 14.6
α-HBDH ($U\,l^{-1}$)	62 ± 5 4	64 ± 7.1	61 ± 6.3
Cholinesterase ($U\,l^{-1}$)	4472 ± 817	4503 ± 859	4481 ± 868
CK ($U\,l^{-1}$)	36 ± 13.7	36.5 ± 13.3	36 ± 13 3
Amylase ($U\,l^{-1}$)	94 ± 15.6	102 2 ± 30.4	94 5 ± 19.7

TABLE V Mean values ± SD of plasma proteins before and after blood passage through the filters examined

Test (unit)	Unfiltered plasma	Filtered plasma	
		Filter 1	Filter 2
Albumin (g %)	3.53 ± 0.18	3.53 ± 0.12	3.49 ± 0.18
α_1-Globulin (g %)	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.02
α_2-Globulin (g %)	0.44 ± 0.09	0.45 ± 0.08	0 45 ± 0.08
β-Globulin (g %)	0.56 ± 0 05	0.59 ± 0.06	0.58 ± 0.04
γ-Globulin (g %)	1.28 ± 0 32	1.23 ± 0.32	1.20 ± 0.32

TABLE VI Mean values ± SD of immunoglobulins and complement factors, before and after blood passage through the filters examined

Test (unit)	Unfiltered plasma	Filtered plasma	
		Filter 1	Filter 2
IgG (mg %)	1123 ± 213 7	1143 ± 236 3	1116 ± 234.1
IgA (mg %)	200 ± 78.2	206 ± 84.3	203 ± 86.5
IgM (mg %)	90 ± 16 5	88 ± 18.9	88 ± 21 1
C_3 (mg %)	52 ± 9.2	52 ± 10.3	52 ± 10.4
C_4 (mg %)	16 ± 3.0	16 ± 3.6	16 ± 3.6

with the exception of a decrease in factor XI of coagulation, which has practically no relevance for the patient's clotting system. The patient is therefore transfused with a type of blood that can treat his or her anaemia without determining side-effects due to altered plasma components. Therefore, the corona treatment, performed on a polyester filter, reduces the priming time without decreasing the efficiency in retaining leucocytes and platelets, prolongs APTT, reduces factor XI, does not induce biochemical modifications and does not alter blood red cells passing through the filter.

Acknowledgement

This research was supported by grants from Istituti Ortopedici Rizzoli, ricerca corrente, area n.1.

References

1. V. MIJOVIC, B BROZOVIC, A. S B HUGHES and T D. DAVIES, *Transfusion* **23** (1983) 30.
2. G SIRCHIA, A PARRAVICINI, P REBULLA, L FATTORI and S MILANI, *Vox Sang.* **38** (1980) 197.
3. A PARRAVICINI and P REBULLA, *Transfusion* **24** (1984) 508
4. O A THOMPSON and J A JAKUBOWSKI, *Blood* **72** (1988) 1.

Received 10 February
and accepted 20 May 1992