Numerical and functional modifications in platelets induced by polyester coated by a hydrophilic polymer

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We evaluated the alterations in number, functionality and release reaction of the platelets contained in plasma, filtered through a polyester filter and coated with a hydrophilic polymer. The alterations in number were examined by counting before and after filtration. The morphological modifications were studied by determining the mean platelet volume. The functional alterations were analysed by a platelet aggregation test, induced by ADP and collagen. The presence of products from the release reaction in filtered plasma was studied using radioimmunoassays of β-thromboglobulin, platelet factor 4 and thromboxane B2. The results obtained showed that the filtration of plasma through the material did not determine a significant platelet adhesion, did not alter the volume or the functionality of the platelets and induced no release reaction.

Keywords: Platelets, polyester, hydrophilic polymer, radioimmunoassay

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The transfusion of platelet concentrates is widely used in the treatment of patients with serious thrombocytopenia. This transfusion can determine hypersensitivity phenomena thought to be caused by leucocytes occurring in the concentrate, to which the patient has developed antibodies due to previous transfusions1-2. To avoid such complications, it was proposed to transfuse platelet concentrates without leucocytes3.

These concentrates can be prepared by using filters, at present made of cotton wool or polyester4, which retain leucocytes and let the platelets through without modifying their functionality and without inducing their activation. However, the filters presently used5 determine a platelet retention ranging from 15 to 20%. Moreover, in our opinion, the functionality of the filtered platelets has not been sufficiently investigated.

The aim of this work is the evaluation of the alterations in number, function and release reaction of the platelets filtered through a polyester filter coated by a hydrophilic polymer. This material could also be used in making filters for platelet concentrates.

The platelet retention determined by such a polymer was evaluated by counting before and after filtration, and the morphological modification was studied by determining the mean platelet volume. The functional alterations were evaluated with Born's platelet aggregation test in plasma before and after filtration. The induction of the release reaction was studied by assaying two platelet proteins (β-thromboglobulin and platelet factor 4) contained in the α-granules and released during the platelet aggregation process. Moreover, thromboxane B2 was assayed. This is a stable metabolite of thromboxane A2, a powerful aggregant agent derived from the arachidonic acid, synthesized by the platelets during their activation.

MATERIALS AND METHODS

Examined material

The material under examination, supplied by Biofil S.R.L. (Cavezzo, Modena, Italy), can be used to manufacture filters for the transfusion of platelet concentrates. It is made of polyester fibres < 3 μm thick coated by a hydrophilic polymer made of partially hydrolysed polyvinylacetate containing polyethylene oxide/polypropylene oxide copolymer block (PEO/PPO) as lateral chains. The coating was carried out by dipping polyester fibres in polymer solution and subsequent evaporation of the solvent. The coating is insoluble in platelet-rich plasma (PRP). The quantity of hydrophilic polymer was 2% of the weight of fibres, evaluated from the polymer solution consumption and the weight of polyester treated. The material was assayed in the form of 7 cm² filters made of woven/non-woven fabric.

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It was not possible to evaluate directly the weight of the coating after the contact with PRP.

**Preparation of platelet-rich plasma**

Venous blood from healthy donors was collected in siliconized tubes, containing as an anticoagulant 3.8% sodium citrate, at a blood:citrate ratio of 9:1. The PRP was obtained by centrifugation at 120g for 5 min at 4°C.

**Contact between PRP and biomaterial**

PRP (20 ml) was filtered through the filter. The filtering time was 60 ± 10 s. Unfiltered PRP was used as a control. The filtered and the unfiltered control plasma were fractioned in aliquots, on which the various tests were carried out.

**Platelet number and volume**

On each sample, platelet number and mean platelet volume were determined by means of Coulter JS counter before and after filtration.

**Platelet aggregation**

Platelet aggregation was carried out before and after filtration. As aggregating agents, ADP (at a concentration of 2 μM) and collagen (at a concentration of 50 μg/ml) were used.

**Assays of β-thromboglobulin, of platelet factor 4 and thromboxane B₂**

A platelet activity-inhibiting solution was added to an aliquot of PRP in the ratio of three parts of PRP and one part of solution, made of 2.5% EDTA, 0.025% 2-chloroadenosine and 7% procaine-HCl. After a 30 min centrifugation at 2000g at 4°C, on the supernatants β-thromboglobulin (β-TG), platelet factor 4 (PF4) and thromboxane B₂ (TXB₂) were assayed.

The concentrations of β-TG and PF4 were determined by competitive radioimmunoassay. For the β-TG assay (Amersham), antigens, labelled with ¹²⁵I-iodine as tracer and rabbit serum as antiserum, were used. The antibody-bound ¹²⁵I-labelled β-TG was separated by precipitation with ammonium sulphate solution. For the PF4 assay (Abbott), antigens, labelled with ¹²⁵I-iodine as tracers and goat serum as antiserum, were used. The antiserum-bound PF4 was separated by precipitation with ammonium sulphate solution.

The concentration of thromboxane B₂ was determined by competitive radioimmunoassay (Amersham). For the assay, TXB₂⁻¹²⁵I-iodotyrosine methyl ester was used as a tracer. Separation of the antibody bound from free fraction was achieved with a second antibody. Before the assay, a chromatographic purification was performed, using columns containing ethyl-bound silica sorbents (Amprep C2 100 mg, Amersham). The columns were conditioned by rinsing with 2.0 ml methanol and 2.0 ml distilled water. The samples of plasma, acidified to pH 3, were applied to the columns; the interferences were removed by rinsing with 5.0 ml distilled water, 5.0 ml 10% ethanol and 5.0 ml hexane. Eluates were collected by passing 5.0 ml methyl formate through the column.

The samples were dried, reconstituted in phosphate-buffer saline and assayed directly. Student’s t test was used to evaluate the statistical significance of the results.

**RESULTS**

The results of platelet counting and mean platelet volume are reported in Table 1. The platelet number shifted from 347.9 ± 59.1 x 10⁶/μl before filtration to 286.1 ± 61.6 after filtration. The percentage of platelets retained by the filter was 16.5 ± 9.01%. The reduction in the number of platelets after filtration was not statistically significant. The mean volume after filtration was unchanged.

The evaluation of platelet aggregation is reported in Table 2. The maximum width induced by ADP in filtered PRP was 80.9 ± 8.5%, while that of unfiltered PRP was 89.1% ± 6.1%. The maximum width of the aggregation induced by collagen was 92.1 ± 6.1% for unfiltered PRP and 91.3 ± 5.5% for filtered PRP. The observed variations did not prove statistically significant. The evaluation of TXB₂ and of the platelet release reaction is shown in Table 3. In the filtered plasma, a decrease in β-TG could be noted, from 565.0 ± 113.1 ng/ml to 484.3 ± 127.0, a reduction of PF4 from 209.2 ± 62.8 ng/ml to 195.2 ± 59.7 and an increase in TXB₂ from 379.7 ± 68.4 pg/ml to 383.3 ± 70.4. The observed variations were not statistically significant.

**DISCUSSION**

Platelet counting highlighted the poor adhesion on the filter. Platelet retention was 16.5%, better than that observed by Bock et al. in filter PL 100 of 25.7%, and similar to the one noticed by Freedman et al. 4, that is 16% for both the polyester and cotton wool filters, and by Bertolini et al., who reported a retention ranging from 12 to 20% for the three commercial filters. Mean platelet volume (MPV) determination permitted to observe that no variation in volume took place after the passage of the platelets through the filter. However, Bertolini et al.

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Table 1: Statistical evaluation of platelet number and mean platelet volume, before and after PRP filtration

<table>
<thead>
<tr>
<th>PRP</th>
<th>Number of tests</th>
<th>Platelet number (x 10⁶/μl)</th>
<th>Mean platelet volume (MPV) (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfiltered</td>
<td>6</td>
<td>347.9 ± 59.1</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>Filtered</td>
<td>6</td>
<td>286.1 ± 61.6</td>
<td>7.3 ± 0.2</td>
</tr>
</tbody>
</table>

Table 2: Statistical evaluation of platelet aggregation, before and after PRP filtration

<table>
<thead>
<tr>
<th>PRP</th>
<th>Maximum width (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADP</td>
<td>Collagen</td>
<td></td>
</tr>
<tr>
<td>Unfiltered</td>
<td>6</td>
<td>89.1 ± 6.1</td>
<td>92.1 ± 6.4</td>
</tr>
<tr>
<td>Filtered</td>
<td>6</td>
<td>80.9 ± 8.5</td>
<td>91.3 ± 5.5</td>
</tr>
</tbody>
</table>
found a reduction of MPV after filtration through three commercial filters

Only a few authors analysed the functionality of filtered platelets. Jaremo et al. studied the aggregation of the platelets of the unfiltered concentrates, while Bock et al. studied the aggregation of the platelets filtered through a modified polyester filter, and found no variation. We chose the aggregation test as index of platelet functionality as well, as this investigates a function necessary for a correct in vivo haemostasis. Filtration through the assayed filter did not determine any variation in the aggregation induced by ADP and collagen. The aggregation test showed that no variation in platelet functionality occurred in the passage through the filter.

The activation of the platelets in the concentrates was examined by Snyder et al. by means of β-thromboglobulin assay and by Jaremo et al. by means of platelet factor 4 assay. The plasmatic concentration of such proteins, specific to the platelets, is correlated to the degree of platelet activation, in this case caused by the passage through the filter. The results obtained by assaying these proteins showed that no platelet activation occurred during the passage through the assayed filter.

The TXB₂ plasma concentration is to be linked to the activation of the metabolic pathway of the arachidonic acid, and therefore to the degree of platelet activation mediated by TXA₂, of which TXB₂ is the stable metabolite. Also the plasmatic levels of this substance remained unchanged after the passage through the examined filter.

CONCLUSIONS

The results of our research enabled us to conclude that the polyester filter treated with the hydrophilic polymer determines a very scanty platelet adhesion, does not alter the functionality of the filtered platelets and does not induce any release reaction.

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