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Influence of storage conditions on the release of growth factors in platelet-rich blood derivatives

DOI 10.1515/cdbme-2016-0069

Abstract: Thrombocytes can be concentrated in blood derivatives and used as autologous transplants e.g. for wound treatment due to the release of growth factors such as platelet derived growth factor (PDGF). Conditions for processing and storage of these platelet-rich blood derivatives influence the release of PDGF from the platelet-bound α-granules into the plasma. In this study Platelet rich plasma (PRP) and Platelet concentrate (PC) were produced with a fully automated centrifugation system. Storage of PRP and PC for 1 h up to 4 months at temperatures between −20°C and +37°C was applied with the aim of evaluating the influence on the amount of released PDGF. Storage at −20°C resulted in the highest release of PDGF in PRP and a time dependency was determined: prolonged storage up to 1 month in PRP and 10 days in PC increased the release of PDGF. Regardless of the storage conditions, the release of PDGF per platelet was higher in PC than in PRP.

Keywords: growth factors; platelet concentrate; platelet derived growth factor; platelet-rich blood derivatives; platelet rich plasma; platelets; storage conditions; thrombocytes.

1 Introduction

Platelet-rich blood derivatives are used as autologous transplants in the field of wound care, general surgery and sports medicine [1]. The main factor for the promotion of healing by applying autologous blood products are growth factors (GF) released by thrombocytes, like platelet derived growth factor (PDGF) [2].

Physiologically growth factors are released after thrombocyte activation due to contact with collagen and other thrombocytes [3]. The initial release of GF due to activation is followed by degradation of the platelets. In vitro contact with foreign surfaces and physical influences such as mechanical (e.g. centrifugation) or thermal (e.g. freezing) stresses additionally activate or disintegrate platelets [4].

A frequently pursued approach in the generation of platelet-rich blood derivatives is preserving the integrity of the platelets to allow a physiological thrombocyte activation after transplantation to the injured area. A contrasting approach is applying blood derivatives rich in released GF to the area in need of a promoted healing effect. It is still unclear which approach results in an optimal global effect [2, 4].

In this context the aim of the present study was to evaluate the influence of storage conditions (temperature and time) on the release of growth factors in autologous platelet-rich blood derivatives.

2 Methods

2.1 Processing of PRP and PC

Venous blood was drawn from healthy donors with 12 ml syringes prefilled with 1 ml of citrate (4% sodium citrate, Duomedica GmbH). The filled syringes (11 ml blood, 1 ml citrate) were connected to empty syringes of equal size with a hose. The connected syringe pair was inserted into a standard laboratory centrifuge (Rotanta 460, Hettich) equipped with a rotor developed for fully automated preparation of blood derivatives. The motorized and sensor controlled system separates the blood fractions within the centrifugation process (see Figure 1).

After the first centrifugation step at 930 g for 5 min plasma and buffy coat are transferred into the connected syringe B until the sensor detects the red blood cells and stops the transfer process. This leads to a donor dependant volume of 5–6 ml of PRP in syringe B.

A second centrifugation step at 475 g for 27 min results in the separation of platelet poor plasma (PPP) and PC within syringe B. The PPP fraction of syringe B is transferred back into syringe A, leaving 1 ml of PC in syringe B.

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During all transfer processes a centrifugation force of 20 g is maintained.

The platelet yield was determined by comparing the number of platelets in the freshly drawn whole blood before preparation and in the blood derivatives after processing. A haematology analyser (KX 21N, Sysmex Europe GmbH) was used.

2.2 Experimental setup

Storage times and temperatures were chosen based on the recommendations stated by the German Medical Association [5]. PRP samples were stored between 1 h and 4 months and PC samples between 1 h and 10 days. The examined storage temperatures were +4°C, +22°C and +37°C for PC and additionally −20°C for PRP.

After storage all samples were brought to room temperature and the amount of released PDGF was determined by ELISA (Human PDGF Duoset ELISA Kit – R&D Systems, Minneapolis, USA).

3 Results

The results were determined as the absolute amount of released PDGF and the amount released per 10⁶ platelets by considering the platelet count.

3.1 Platelet rich plasma

The average yield of platelets in PRP gained from freshly drawn venous blood samples was 97.2 ± 7.8% with a platelet concentration of 402 ± 24 × 10³ plt/µl (1.75 ± 0.13 fold compared to the unprocessed whole blood sample: n = 15).

The content of PDGF in PRP was measured and averaged over 3 donors (see Figure 2). PDGF content increased with storage times within the 1st month and subsequently slightly decreased within the following 3 months, regardless of the storage temperature. For all storage times the highest values were reached at −20°C storage temperature.

In addition the PDGF release per 10⁶ platelets was calculated based on the number of platelets measured in the samples before storage (see Table 1). An increase of PDGF release within the 1st month of storage and decrease within the following 3 months, regardless of the storage temperature was also evident here. The highest overall rate was reached after storage of PRP for 1 month at −20°C with 33 pg per 10⁶ platelets.

3.2 Platelet concentrate

The average yield of platelets in PC gained from freshly drawn whole blood samples was 69.4 ± 21.3% with a platelet concentration of 1528 ± 477 × 10³ plt/µl –20°C

<table>
<thead>
<tr>
<th>Time</th>
<th>−20°C</th>
<th>+4°C</th>
<th>+22°C</th>
<th>+37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>6.7 ± 6.3</td>
<td>2.6 ± 1.0</td>
<td>1.9 ± 0.9</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>24 h</td>
<td>16.6 ± 2.3</td>
<td>7.1 ± 0.7</td>
<td>3.2 ± 1.0</td>
<td>4.7 ± 1.7</td>
</tr>
<tr>
<td>48 h</td>
<td>16.3 ± 1.6</td>
<td>7.4 ± 1.0</td>
<td>5.5 ± 1.1</td>
<td>5.9 ± 1.4</td>
</tr>
<tr>
<td>1 month</td>
<td>30.6 ± 2.0</td>
<td>25.2 ± 3.9</td>
<td>25.3 ± 4.0</td>
<td>26.9 ± 1.1</td>
</tr>
<tr>
<td>4 months</td>
<td>22.0 ± 0.5</td>
<td>21.7 ± 0.6</td>
<td>20.3 ± 0.6</td>
<td>–</td>
</tr>
</tbody>
</table>
(6.64 ± 2.13 fold compared to the whole blood sample: n = 18).

The content of PDGF in PC was measured and averaged over 3 donors (see Figure 3). PDGF content increased with storage time within 10 days of storage, regardless of the storage temperature. PDGF release within the first 72 h was comparable for all storage. Chilled storage at +4°C resulted in the highest PDGF content within the following 7 days.

The PDGF release per 10⁶ platelets was calculated based on the number of platelets measured before storage (see Table 2). The highest amount of PDGF per 10⁶ platelets was reached at +22°C for all storage times within the first 72 h and at +4°C in the following 7 days. The release increased with prolonged storage time for each temperature. The highest overall rate was reached in a PC sample from donor 1 stored for 10 days at 4°C with a calculated value of 670 pg per 10⁶ platelets.

4 Discussion

Platelet-rich blood derivatives contain multiple growth factors with positive effects on the treatment of injured areas [2]. As PDGF is one of the most important and well-characterized growth factors it was chosen as a representative and marker in this study. Our aim was to evaluate the release of PDGF from the platelet-bound α-granules into the plasma induced by storage conditions, regardless of the intended therapeutic approach. Nevertheless the findings provide information for the objective of low platelet activation as well as maximised release of growth factors in the blood derivatives.

The first influence on PDGF release in blood derivatives is the production process. We used a fully automated system to generate PRP and PC leading to a highly reproducible process compared to standard preparation processes comprising manual steps. Thus reasons for variations of the platelet yield can be reduced to donor dependent differences.

In both PRP and PC the increased release of PDGF with storage time can be explained by activation induced by mechanical and thermal stress [4] followed by degranulation and disintegration of thrombocytes. Disintegration of blood cells leads to false platelet measurements in haematology analysers [6]. Thus, variations of the platelet number after storage might be considered as an indicator for degradation and disintegration of the thrombocytes. This results in loss of physiological activity and integrity of the platelets as desired in some therapeutic approaches. In PRP only small changes were detected in the number of platelets measured within the first 48 h of storage above +4°C indicating that mainly platelet activation and not disintegration caused the PDGF release. For prolonged and frozen storage high fluctuations of the platelet count were determined in PRP and disintegration of the platelets is assumed. In PC this was already observed in the first 72 h of storage.

4.1 Platelet rich plasma

In PRP nearly no difference was noticed in the PDGF content between the tested storage temperatures after 1 month of storage. Storage of the platelets above +4°C lead to a continuous release of PDGF almost levelling with the amount released at −20°C after 1 month. The following decrease of the PDGF content at all temperatures might be due to denaturation of the released growth factors. The theoretically possible maximum level of 60 pg per 10⁶ platelets bound in the α-granules [7] was not reached in the blood derivatives.

A further observation in the frozen samples of PRP which might explain the higher values of PDGF was a very homogenous composition with no apparent phase
separation. Due to the static storage without agitation the blood derivatives stored at +4°C, +22°C, and +37°C had visible phase separation. This could result in variations of the pH value and thus in denaturation of proteins explaining the lower PDGF content. By quickly freezing the blood derivatives stored at −20°C no phase separation was possible despite the equally static storage conditions.

4.2 Platelet concentrate

In PC the thrombocytes are more densely packed than in PRP due to the higher concentration. Contact activation of the densely packed platelets needs to be taken into account. Also the greater physical influence of the two step centrifugation process has an effect on the release of growth factors.

The PDGF concentration in all PC samples was higher than in PRP, which could be explained by the higher platelet content. However higher values were also detected when comparing the PDGF amounts standardized to the platelet count. Within the first 10 days of storage the amount of PDGF released per 10⁶ platelets in PC was higher than the overall maximum in PRP, regardless of the storage conditions. Furthermore the release within 72 h in PC already equalled the amount released in PRP within 1 month of storage.

This indicates that PDGF release in PC is distinctly accelerated compared to PRP and might be due to the stronger activation during production and contact activation. The values of PDGF per 10⁶ platelets are also closer to the maximum possible physiological level of 60 pg per 10⁶ platelets. In addition storage at −20°C could be a promising condition for an even higher yield and complete release of growth factors in PC.

5 Conclusion

Storage of platelet-rich blood derivatives resulted in an increased release of PDGF from the platelet-bound α-granules into the plasma with storage time up to 1 month in PRP and 10 days in PC, regardless of the storage temperature. PDGF release per platelet in PC was distinctly accelerated compared with PRP and almost reached the physiologically possible level of 60 pg per 10⁶ platelets. Highest PDGF levels were reached in chilled or frozen storage with accelerated PDGF release in PRP at −20°C.

The findings provide information for the objective of low platelet activation as well as maximised release of growth factors in blood derivatives due to storage conditions.

Acknowledgment: The authors are grateful to Andreas Hettich GmbH & Co.KG for the project support.

Author’s Statement

Research funding: The author state no funding involved. Conflict of interest: Authors state no conflict of interest. Material and methods: Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee.

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