Platelet-Rich Plasma Induces Peripheral Blood Mononuclear Cell Migration in a Dose-Dependent Manner

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INTRODUCTION:

Advances in the understanding of tissue repair mechanisms have revealed the essential role of cytokines and growth factors in coordinating the events of wound healing at the site of injury. As a result, platelet-rich plasma therapies are emerging as a potent source of possible regulatory factors to both modify and enhance tissue healing. Once a tissue is injured, the inflammatory response recruits local and peripheral cells to the site by a series of complex signaling cascades and chemoattractants. We hypothesize that a number of the chemotactic factors present in platelet rich plasma aid in recruiting and activating peripheral blood mononuclear cells (PMBCs), namely lymphocytes, monocytes, and macrophages, to the wound site. To explore potential mechanisms for platelet effects on wound healing, including the stimulation of cellular invasion into the wound site, we examined PBMC migration into increasing concentrations of autologous platelet-rich plasma (PRP) via a Boyden chamber migration assay.

METHODS:

Human blood (1.70E+08 platelets/mL) was collected in acid citrate dextrose (ACD) and centrifuged to isolate a plasma/platelet fraction, which was used to make various concentrations of autologous PRP. PBMCs were isolated from the red blood cell/leukocyte fraction by Ficoll-Hypaque density gradient centrifugation. Isolated PBMCs were labeled with the fluorescent dye Calcein AM and resuspended in 1X PBS/0.1% FBS for the assay. Chemotaxis of PBMCs into increasing concentrations of PRP was quantified using a 96-well ChemoTx chemotaxis chamber with polycarbonate filters with a 5 μm pore size. The wells in the microplate were filled with 300 μl of PRP, PPP, or 1X PBS/0.1% FBS (n=16 for each group). 50 μl of a cell suspension of fluorescently labeled PBMCs was placed directly onto the filter sites. After incubation for 3h at 37°C and 5% CO2, the microplate was read using a multi-well fluorescent plate reader. Single factor ANOVA and two-tailed t-Test analyses were performed to test the hypothesis that using different concentrations of PRP as a chemoattractant resulted in statistically significant differences in PBMC migration.

RESULTS:

The effect of platelet concentration on migration of PBMCs in vitro was tested using a Boyden chamber migration assay. Significant differences (p = 0.0014) in migration of PBMCs into preparations of PRP with various concentrations of platelets were found.

PBMC Migration vs. PRP Concentration

<table>
<thead>
<tr>
<th>Chemoattractant</th>
<th>Platelet Concentration (PLT/mL)</th>
<th>Migration (PBMCs/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>0.00</td>
<td>9.36E+05</td>
</tr>
<tr>
<td>PPP</td>
<td>1.33E+08</td>
<td>2.31E+06</td>
</tr>
<tr>
<td>3X PRP</td>
<td>5.42E+08</td>
<td>3.26E+06</td>
</tr>
<tr>
<td>5X PRP</td>
<td>8.92E+08</td>
<td>3.39E+06</td>
</tr>
</tbody>
</table>

Figure 1. Platelet concentration in chemoattractant, and migration results. Migration is represented as mean concentration of PBMCs after migration with n = 16 for each group.

DISCUSSION:

While there has been much recent interest in the use of platelet-rich plasma in stimulating wound healing, less is known about its mechanism of action. The data reported here suggest that there is a direct relationship between the concentration of platelets in the provisional scaffold and the ability of that scaffold to recruit circulating inflammatory cells. The functional effects of this dependency require further study. In addition, it is unknown as yet which of the myriad of cytokines released by platelets is responsible for the chemoattractant nature of the platelet-clot; further study to delineate this is also warranted.

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