Endogenous G-CSF and CD34+ cell mobilization after acute myocardial infarction

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Abstract

Background: Several reports showed an increase of CD34+ stem/progenitor cell count early after an acute myocardial infarction (AMI), suggesting a contribution of bone marrow cells in myocardial regeneration after the acute event. Nevertheless, at present plasma mediators of CD34+ cell mobilization from bone marrow to peripheral blood in patients with AMI are poorly understood. Aim of our study was to establish the impact of different well-known mobilizing cytokines on spontaneous stem cell mobilization in patients with different ischemic heart syndromes, such as the AMI and the chronic stable angina (CSA), compared to healthy controls.

Methods: In 16 patients with AMI, 18 with CSA and 22 healthy blood donors the concentration of CD34+ cells, and mobilizing cyokines (G-CSF, SCF, VEGF, SDF1-alpha) were assessed.

Results: The peak number of circulating CD34+ cells in AMI patients (8.58 ± 2.08 cells/µl) was higher than that observed in patients with CSA (3.41 ± 0.56 cells/µl, p = 0.0061) or in healthy controls (2.18 ± 0.35 cells/µl, p < 0.001). However endogenous G-CSF was significantly higher in the serum of patients with AMI compared to CSA patients and to controls and in CSA patients compared to controls. Interestingly, as regards VEGF, while this cytokine was increased in AMI with respect to control and CSA group, the latter showed a significantly lower concentration with respect to controls. Finally SDF-1 alpha was higher in AMI patients with respect to controls. CD34+ cells were significantly correlated to G-CSF (directly) and to SCF (inversely) in patients with AMI.

Conclusion: In the present study, we have demonstrated for the first time that the spontaneous mobilization of CD34+ cells into the peripheral blood of patients with AMI is significantly correlated to endogenous G-CSF. Considering recent data suggesting a potential favourable effect of circulating CD34+ cells on left ventricular function, the present evidence of a correlation between endogenous G-CSF and CD34+ cell levels supports the pharmacological administration of G-CSF as a non-invasive option for regeneration of myocardial tissue after AMI.

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Keywords: Acute myocardial infarction; G-CSF; CD34+ cells

1. Introduction

The CD34 antigen is considered the common antigen for haematopoietic stem/progenitor and endothelial progenitor cells. Several reports showed an increase of CD34+ cell count early after an acute myocardial infarction (AMI) [1], potentially suggesting a contribution of bone marrow cells
in myocardial regeneration after the acute event [2]. Effectively, we recently demonstrated that CD34+ cell mobilization, especially when persistent, is favourably correlated to post-infarction left ventricular remodelling [2]. Nevertheless, at present plasma mediators of CD34+ cell mobilization from bone marrow to peripheral blood in patients with AMI are poorly understood. Moreover we cannot exclude that mechanisms leading to CD34+ cell mobilization in the acute patients could be different with respect to those who tonically regulate CD34+ cells release in chronic coronary artery disease and in normal healthy subjects.

Aim of our study was to establish the impact of different well-known mobilizing cytokines on spontaneous stem cell mobilization in patients with different ischemic heart syndromes, such as the AMI and the chronic stable angina (CSA), compared to healthy controls.

2. Patients and methods

2.1. Patients’ characteristics

We consecutively enrolled 16 patients with AMI, 18 with CSA and as controls, 22 healthy blood donors without overt heart disease and/or major cardiovascular risk factors (diabetes, smoking, hypertension, hypercholesterolemia and familial history) (Table 1). Peripheral blood samples were obtained 1, 3, 5 and 7 days following AMI.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>AMI Mean ± S.E.M.</th>
<th>CSA Mean ± S.E.M.</th>
<th>Healthy controls Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59.4 ± 3.4</td>
<td>66.3 ± 1.1</td>
<td>37.6 ± 2.5</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>46.5 ± 3.5</td>
<td>65 ± 5</td>
<td>Normal</td>
</tr>
<tr>
<td>WMSI</td>
<td>1.59 ± 0.13</td>
<td>1</td>
<td>Normal</td>
</tr>
<tr>
<td>CPK (UI/L)</td>
<td>3780 ± 1266</td>
<td>118 ± 24</td>
<td>80 ± 45</td>
</tr>
<tr>
<td>TNt (ng/ml)</td>
<td>7.86 ± 1.52</td>
<td>&lt;0.01</td>
<td>N/A</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>325 ± 19</td>
<td>283 ± 11</td>
<td>238 ± 9</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>13.2 ± 3.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>WBC (cells/μl)</td>
<td>12700 ± 888</td>
<td>7347 ± 327</td>
<td>6381 ± 226</td>
</tr>
</tbody>
</table>

2.2. Measurement of CD34 cell count

The frequency of CD34+ cells was quantified in un-fractionated peripheral blood samples using an established, single-platform enumeration method. Samples were collected on days 1, 3, 5 and 7 following AMI. A single sample was obtained in CSA patients and in healthy controls. Briefly, 100 μl of PB was labelled with saturating amounts of PE-conjugated anti-CD34 and FITC-conjugated anti-CD45 mAbs for 30 min on ice. Following ammonium chloride erythrocyte lysis, cells were washed with PBS–1% BSA and re-suspended in PBS containing 20 μg/ml 7-amino-actinomycin-D. 7-AAD+ events were excluded from the analysis.

Samples were run through a FACScan® flow cytometer (BD) with standard equipment. Antigen expression was quantified both in terms of percentage of positive cells and in terms of mean fluorescence intensity (MFI), using the CellQuest® software (BD). The probability of significant differences between the distribution of test and control histograms was calculated with the Kolmogorov–Smirnov test. Details on instrument settings and data analysis were published elsewhere [2].

2.3. Analysis of serum cytokine levels

Peripheral blood samples were centrifuged for 15 min at 4000 rpm. Sera were aliquoted and stored at −80 °C until use. The serum levels of granulocyte-colony stimulating factor (G-CSF), stem cell factor (SCF), vascular endothelial growth factor (VEGF) and stromal derived factor (SDF)-1 alpha were quantified by ELISAs (Quantikine Immunoassay, R&D Systems).

2.4. Statistical analyses

All results were presented as mean ± standard error of the mean (S.E.M.). Because of the small sample size non-parametric tests (Mann–Whitney or Kruskal–Wallis tests) were used. Correlations were evaluated by Spearman rank analysis. P value <0.05 was considered significant.

3. Results

3.1. Circulating CD34+ cells increase early after AMI

We first determined whether cells expressing the stem cell-associated antigen CD34 were increased in the peripheral blood of patients with AMI. To this end, peripheral blood samples were collected on days 1, 3, 5 and 7 following AMI. According to our previous study [2] in AMI patients the number of circulating CD34+ cells was similar on days 1, 3, 5 and 7 (7.77 ± 2.77, 4.14 ± 1.15, 3.91 ± 0.50 and 3.73 ± 0.43 cells/μl, respectively; p = 0.89). The peak number of circulating CD34+ cells in AMI patients...
(8.58 ± 2.08 cells/µl) was higher than that observed in patients with CSA (3.41 ± 0.56 cells/µl, p = 0.0061) or in healthy controls (2.18 ± 0.35 cells/µl, p < 0.001). Furthermore, the number of CD34+ cells in patients with CSA was higher than in controls (p = 0.05).

3.2. Release of stem/progenitor cells-mobilizing cytokines early after AMI

We assessed the serum release of prototypical cytokines involved in stem cell mobilization. AMI patients showed a variable release of the 4 assessed cytokines after the acute event without any statistically significant between the different time points. Nevertheless VEGF showed a significant trend to an increased concentration from the day 1 to the day 7 (p for trend = 0.01) (Fig. 1; Table 2). However peak level of endogenous G-CSF was significantly higher in the serum of patients with AMI compared to CSA patients and to controls and in CSA patients compared to controls. Interestingly, as regards VEGF, while this cytokine was increased in AMI with respect to control and CSA group, the latter showed a significantly lower concentration with respect to controls. Finally SDF-1 alpha was higher in AMI patients with respect to controls (Table 2; Figs. 1 and 2).

CD34+ cells were significantly correlated to G-CSF (directly) and to SCF (inversely) in patients with AMI; of note neither G-CSF nor the other cytokines were significantly correlated to white blood cell count, suggesting that these cytokines were not the mediators of post-infarction leukocytosis (for G-CSF: r = 0.12, p = 0.40; for SCF: r = -0.21, p = 0.13; for VEGF r = 0.04, p = 0.76; for SDF-1 alpha: r = -0.08, p = 0.55). Differently, CD34+ cell concentration was not correlated significantly to any of

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF (pg/ml)</td>
<td>47.85 ± 117.8</td>
<td>45.93 ± 97.65</td>
<td>21.85 ± 12.91</td>
<td>55.22 ± 102.0</td>
<td>0.59</td>
</tr>
<tr>
<td>SCF (pg/ml)</td>
<td>563.8 ± 225.1</td>
<td>538.6 ± 186.2</td>
<td>538.1 ± 172.8</td>
<td>522.4 ± 205.0</td>
<td>0.93</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>81.23 ± 64.80</td>
<td>93.33 ± 67.92</td>
<td>110.0 ± 103.7</td>
<td>175.4 ± 111.8</td>
<td>0.17</td>
</tr>
<tr>
<td>SDF-1 alpha (pg/ml)</td>
<td>2232 ± 758.6</td>
<td>2536 ± 747.9</td>
<td>2642 ± 520.6</td>
<td>2694 ± 477.1</td>
<td>0.37</td>
</tr>
</tbody>
</table>
the tested cytokines in CSA patients, while in controls was significantly and directly correlated to VEGF levels. Finally G-CSF reassessed in AMI patients at 1-year follow-up was comparable to healthy controls (10.36 ± 2.18 pg/ml), suggesting that the early increase of G-CSF levels was specific for the early phases of AMI. Collectively, cytokine studies pointed to endogenous G-CSF as a potential determinant of post-infarction stem/progenitor cells mobilization (Table 3; Figs. 3–5).

4. Discussion

The injection of stem/progenitor cells has been recently proposed as potential therapeutic option for patients with AMI [3,4]. Nevertheless, increase of circulating CD34+ EPCs after AMI is a well-documented phenomenon potentially influencing left ventricular function in the post-infarction setting [2] and in congestive heart failure [5]. Although it is likely that the origin of circulating CD34+ is represented by the bone marrow and it might be postulated that ischemic tissues might release inflammatory or angiogenic cytokines, however at present the circulating mediators of CD34+ cell mobilization are not completely known. In the present study, we have demonstrated for the first time that the spontaneous mobilization of CD34+ cells into the peripheral blood of patients with AMI is significantly correlated to endogenous G-CSF. G-CSF is a well-known potent mobilizer of CD34+ cells into peripheral blood and is currently widely used for transplantation of haematopoietic progenitor cells instead of the whole bone marrow in current haematological practice. For its characteristics of tolerability, safety and efficacy on CD34+ cell mobilization several studies are currently ongoing to test the possible beneficial effect on post-infarction left ventricular function [6,7]. Considering our previous data [2] suggesting a favourable effect of CD34

Table 3

Increased concentration of G-CSF in AMI patients; reduced concentration of VEGF in CSA patients

<table>
<thead>
<tr>
<th></th>
<th>AMI</th>
<th>CSA</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF (pg/ml)</td>
<td>92.88 ± 145.1 a</td>
<td>28.67 ± 46.68 b</td>
<td>10.64 ± 5.551</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SCF (pg/ml)</td>
<td>598.6 ± 204.4 c</td>
<td>556.3 ± 218.9 d</td>
<td>597.1 ± 165.7</td>
<td>0.48</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>178.8 ± 110.9 e</td>
<td>42.67 ± 28.44 f</td>
<td>92.59 ± 69.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SDF-1 alpha (pg/ml)</td>
<td>2931 ± 529.8 g</td>
<td>2753 ± 469.0 h</td>
<td>2564 ± 474.1</td>
<td>0.12</td>
</tr>
</tbody>
</table>

a Versus CSA: p = 0.03; versus controls: p < 0.0001.
b Versus controls: p = 0.05.
c Versus CSA: p = 0.44; versus controls: p = 0.81.
d Versus controls: p = 0.24.
e Versus CSA: p < 0.0001; versus controls: p = 0.005.
f Versus controls: p = 0.002.
g Versus CSA: p = 0.37; versus controls: p = 0.04.
h Versus controls: p = 0.28.
Fig. 3. Correlations between CD34⁺ cell count and mobilizing cytokines at day 1, 3, 5 and 7 in patients with AMI (for graphical reasons data are reported in log scale).

Fig. 4. Correlations between CD34⁺ cell count and mobilizing cytokines in patients with CSA (for graphical reasons data are reported in log scale).
cell mobilization of left ventricular function, the present evidence of a significant correlation between endogenous G-CSF and CD34+ cell levels supports consistently that pharmacological administration of G-CSF could be a non-invasive option for regeneration of myocardial tissue after AMI.

Importantly we found that mechanisms of mobilization are probably different in different clinical settings. In fact CD34+ cell level was significantly correlated to VEGF levels in healthy controls while in patients with CSA was correlated to none of the tested cytokines. Considering that VEGF absolute levels appeared significantly reduced in CSA patients with respect to controls, it might be speculated that CD34+ cell levels are tonically controlled by VEGF levels in normal subjects but when a potent stimulus, such as the acute ischemia of the AMI, occurs the effect of acutely released cytokines, as G-CSF, overwhelms the VEGF effect, particularly if we consider that myocardial release of VEGF needs several hours and days after the acute event to be apparent [8]. This is confirmed by the significant trend towards an increased concentration of VEGF seen in patients with AMI. Nevertheless, this finding could support other intriguing speculations: the progressively increased concentration of VEGF in patients with AMI could play a possible role of neo-angiogenesis mediated by the mobilization of committed endothelial progenitor cells [1] in the mechanisms of myocardial healing, while its depletion observed in the group of patients with CSA could partially account for the scarce neo-vascularization capacity observed in patients with chronic ischemic heart disease [9]. Taken together all these data support the therapeutic utilization of intramyocardial VEGF gene transfer [10] in patients with chronic ischemic heart disease.

Recently, considerable attention has been devoted to the role of the chemokine stromal cell-derived factor-1 alpha (SDF-1 alpha) and its receptor CXCR4 in the trafficking and homing of human stem/progenitor cells. Interestingly, locally delivered SDF-1a contributes to the recruitment of EPCs and thus augments vasculogenesis in ischemic tissues [11]. Furthermore, SDF-1 alpha is transiently up-regulated at the mRNA level early after myocardial infarction and might contribute to intraslesional stem/progenitor cells homing [12]. These observations suggest that therapeutic strategies focused on stem/progenitor cell mobilization should be initiated early after AMI in order to take advantage of the rapid up-regulation of SDF-1 alpha expression within the infarct zone. Accordingly, we found significantly higher levels of SDF-1 alpha in AMI patients compared to healthy controls. Nevertheless, as confirmed from the lack of correlation between CD34+ cell and SDF-1 alpha levels, SDF-1 alpha seems to be a prevalently local mediator for engraftment of stem/progenitor cell rather than a systemic one suggesting that other systemic effectors, such...
as G-CSF, are needed to mobilize stem/progenitor cells towards the ischemic myocardium. This is in general agreement with our previous findings in which we demonstrated that G-CSF induces CD34⁺ cells to express high levels of the CXCR4 receptor [13], the receptor for the SDF-1 alpha, and that circulating CD34⁺ cells in patients with AMI showed high levels of CXCR4 receptor suggesting their potential to home in the microenvironment of the injured myocardium where SDF-1 alpha levels are expected to be up-regulated [2]. Collectively our data underline the pivotal role of the interplay between the systemic mobilizing effect of G-CSF and the local contribution to engraftment of SDF1-alpha.

4.1. Limitations of the study

The limited sample size could have partially influenced the results of our study; in particular, the potential influence of other important cytokines, such as VEGF, could be underestimated for an underpowering. Moreover, despite correlation between CD34⁺ cell count and endogenous G-CSF levels is significant, nevertheless it does not prove necessarily a cause–effect relationship, especially if we consider that $R^2$ is quite low and so that other factors, not yet completely understood, can play an important role in stem/progenitor cell mobilization.

5. Conclusions

Endogenous G-CSF is significantly increased in the acute phases of myocardial infarction and is directly correlated to CD34⁺ cell levels, suggesting its pivotal role in the CD34⁺ stem/progenitor cell mobilization. Considering recent data suggesting a potential favourable effect of circulating CD34⁺ cells on left ventricular function, G-CSF could be a non-invasive option to ameliorate post-infarction remodelling by an increased mobilization of stem/progenitor cells.

References