Effect of Atrial Fibrillation on Hematopoietic Progenitor Cells

A Novel Pathophysiological Role of the Atrial Natriuretic Peptide?

Andreas Goette, MD*; Kathleen Jentsch-Ullrich, MD*; Uwe Lendeckel, PhD; Christoph Röcken, MD; Mahmood Agbaria, MS; Angelo Auricchio, MD; Martin Mohren, MD; Astrid Franke, MD; Helmut U. Klein, MD

Background—Injury to the heart causes hematopoietic progenitor cells (HPCs) to migrate to the site of damage and to undergo cell differentiation. Studies suggest that myocardial progenitor cells invade atrial tissue. So far it is unclear, however, whether an atrial disease per se affects circulating HPCs.

Methods and Results—Seventeen patients with persistent atrial fibrillation (persistAF), 12 with paroxysmal AF (paroxAF), and 17 matched patients with sinus rhythm (SR) were studied. HPCs (CD34+/CD117+) were quantified with the use of a fluorescence-activated cell sorter; stromal cell–derived factor-1α (SDF-1α), vascular endothelium growth factor (VEGF), and atrial natriuretic peptide (ANP) were determined by immunoassays. In patients with persistAF, blood samples were obtained before as well as 10 minutes, 24 hours, and 48 hours after electrical cardioversion. CD34+HPCs (AF, 7.0±2.3×10^3/mL versus SR, 5.0±1.6×10^3/mL; P<0.01) were increased during persistAF only. Highest SDF-1α levels were also observed during persistAF. Successful and unsuccessful cardioversion decreased CD34+HPCs temporarily (7.0±2.3×10^3/mL versus 24 hours: 5.0±1.5×10^3/mL; P<0.05). Forty-eight hours after successful cardioversion, SDF-1α and CD34+HPC levels started to decline, reaching control levels after 59±19 days. CD34+/CD117+ and VEGF levels, however, were increased by DC energy but not by AF. ANP levels correlated with CD34+HPC (r=0.76; P<0.01) and SDF-1α (r=0.56; P<0.01). HPCs from patients with AF had a greater tendency to differentiate into cells expressing (cardio)myocyte markers ANP and myocyte enhancer factor-2.

Conclusions—PersistAF appears to increase the potential of HPCs for (cardio)myogenesis. Restitution of CD34+HPC levels, mediated by SDF-1α and possibly ANP, occurs within several weeks after successful cardioversion.

Key Words: arrhythmia ♦ blood cells ♦ cardioversion ♦ fibrillation ♦ natriuretic peptides

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njury to the heart causes hematopoietic progenitor cells (HPCs) to migrate to the site of damage and to undergo progenitor cell differentiation.1,2 Recent studies suggest that multipotent cells can transdifferentiate into cardiomyocytes.1,3 Interestingly, Quaini et al2 have demonstrated a high proportion of progenitor cells, especially in atrial tissue. It is not known so far whether atrial fibrillation (AF), which is known to cause structural atrial changes, affects HPCs. The purpose of the present study was to quantify CD34+ and CD34+/CD117+ HPCs, stromal cell–derived factor-1α (SDF-1α), vascular endothelium growth factor (VEGF), and atrial natriuretic peptide (proANP 1-98) in patients with persistent (persistAF) and paroxysmal AF (paroxAF). In addition, the effect of electrical cardioversion on HPCs and HPC factors was determined.

Methods

Patients
Forty-six patients were included in the study. Seventeen patients had persistAF (≥3 months) and were scheduled for cardioversion. Twelve patients had paroxAF; 17 patients were in sinus rhythm (SR) and served as age- and sex-matched control subjects (Table). All patients were in New York Heart Association classes I and II. Patients with AF had been anticoagulated with warfarin for at least 4 weeks. Antiarrhythmic drugs were started 72 hours before cardioversion (Table). The study protocol was approved by the Ethics Committee of the University Hospital Magdeburg, Germany. All patients gave written informed consent.

CD34+/CD117+ Analysis
Blood samples (5 to 20 mL) were drawn with an 18-gauge needle (Becton Dickinson [BD]) from a cubital vein. In patients with persistAF, blood samples were taken before as well as 10 minutes, 24
### Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Sinus Rhythm</th>
<th>Persistent AF</th>
<th>Paroxysmal AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>17</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Age, y</td>
<td>48 ± 18</td>
<td>53 ± 6</td>
<td>60 ± 15</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>12/5</td>
<td>12/5</td>
<td>5/7</td>
</tr>
<tr>
<td>History of AF, mo</td>
<td>...</td>
<td>11 ± 6</td>
<td>22 ± 23</td>
</tr>
<tr>
<td>CAD, % (n)</td>
<td>12 (2)</td>
<td>18 (3)</td>
<td>25 (3)</td>
</tr>
<tr>
<td>MI, % (n)</td>
<td>12 (2)</td>
<td>6 (1)</td>
<td>0 (0)</td>
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<tr>
<td>LVEF, %</td>
<td>56 ± 16</td>
<td>63 ± 8</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>LAD, cm</td>
<td>4.3 ± 1.2</td>
<td>4.6 ± 0.4*</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Hypertension, % (n)</td>
<td>65 (11)</td>
<td>88 (15)</td>
<td>58 (7)</td>
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<tr>
<td>Diabetes, % (n)</td>
<td>35 (6)</td>
<td>25 (9)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>HLP, % (n)</td>
<td>71 (12)</td>
<td>53 (9)</td>
<td>25 (3)</td>
</tr>
<tr>
<td>β-Blockers, % (n)</td>
<td>47 (8)</td>
<td>65 (11)</td>
<td>42 (5)</td>
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<tr>
<td>Calcium antagonists, % (n)</td>
<td>18 (3)</td>
<td>29 (5)</td>
<td>25 (3)</td>
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<tr>
<td>Class I AA, % (n)</td>
<td>...</td>
<td>29 (5)</td>
<td>25 (3)</td>
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<tr>
<td>Class III AA, % (n)</td>
<td>...</td>
<td>71 (12)</td>
<td>8 (1)</td>
</tr>
<tr>
<td>ACE inhibitors, % (n)</td>
<td>53 (9)</td>
<td>59 (10)</td>
<td>42 (5)</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; MI, history of myocardial infarction; LVEF, left ventricular ejection fraction; LAD, left atrial diameter; HLP, hyperlipidemia; and class I/III AA, class I/III antiarrhythmic drugs. Numbers in parentheses represent the absolute number. *P < 0.05, persistent vs paroxysmal AF.

### Immunoassays

Ice-cooled EDTA blood was used to determine human SDF-1α, VEGF, and proANP(1-98) plasma levels. Blood samples were centrifuged at 1000g within 30 minutes after collection. Human SDF-1α, VEGF, interleukin (IL)-6, and IL-10 (R&D Systems) as well as proANP(1-98) (Biomedica) were determined by commercially available immunoassays according to the manufacturer’s specifications.

### Cell Culture and Immunostaining

In 8 patients (4 persistAF, 4 SR) mononuclear cell fraction was separated with Ficoll-Paque Plus (Amer sham Pharmacia) centrifugation and then cultured at a concentration of 1 × 10^6/mL in Iscove’s modified Dulbecco’s medium with 2% fetal bovine serum (Stem Cell Technologies) and methylcellulose (Method CF H4434). Methods for CFU-GM assays were previously described. After 4 days in culture, cells were transferred onto slides by cytopsin centrifugation, fixed in methanol-acetone-formalin-mixture, and stained for the myocyte enhancer factor (MEF-2) (Santa Cruz), ANP (Peninsula Laboratories), and CD31 (DAKO), by use of the APAAP method (Dako En Vision System).7

### Electrical Cardioversion

Electrical cardioversion was performed under sedation with intravenous midazolam (2 mg) and etomidate (0.2 mg/kg). Three hundred sixty–Joule shocks (LIFEPAK 10, Physio-Control) were applied to the patients by self-adhesive skin electrodes (Physio-Control) in an anterior-posterior position.

### Statistical Analysis

All values are expressed as mean ± SD. ANOVA analyses were used to assess differences between groups and within groups. A value of *P* < 0.05 was considered statistically significant.

### Results

#### Effect of AF and Electrical Cardioversion

Baseline CD34^+ levels were significantly elevated in patients with persistAF (7.0 ± 2.3 × 10^3/mL versus SR, 5.0 ± 1.6 × 10^3/mL; *P < 0.01*). In contrast, there was no difference between paroxAF and SR (3.6 ± 1.3 × 10^3/mL versus SR, 5.0 ± 1.6 × 10^3/mL; *P = NS*) (Figure 1A). CD34^+/CD117^+ levels were not different (persistAF, 0.19 ± 0.12 × 10^3/mL; paroxAF, 0.14 ± 0.11 × 10^3/mL; SR, 0.26 ± 0.21 × 10^3/mL; *P = NS*). Heavy sedation alone did not change CD34^+ or CD34^+/CD117^+ levels (data not shown).

Fourteen of 17 patients (82%) with persistAF were successfully converted to SR [CV(+); cardioversion was unsuccessful [CV(−)] in the remaining 3 of 17 patients (18%). None of the 14 patients with CV(+) had a recurrence of AF during follow-up. Ten minutes after application of 1 DC shock (mean, 1; range, 1 to 3; total mean energy, 487 ± 253 J), the amount of CD34^+HPCs decreased in all patients (Figure 1A). Interestingly, the decrease occurred regardless of the outcome of cardioversion [CV(+), 5.5 ± 1.7 × 10^3/mL versus CV(−), 5.0 ± 0.9 × 10^3/mL; *P = NS*]. The decrease in CD34^+HPCs was greater in 4 patients who received more than 1 shock (1 DC, −17 ± 14% versus ≥ 2 DC, −45 ± 24%; *P < 0.05*). After the transient drop, CD34^+ levels increased to baseline within 48 hours (24 hours, 5.0 ± 1.5 × 10^3/mL versus 48 hours, 7.4 ± 2.7 × 10^3/mL; *P < 0.05*) in all patients. After CV(+), CD34^+ levels decreased during follow-up (−32 ± 23%; *P < 0.05*), reaching levels similar to SR [CV(+), 5.6 ± 2.2 × 10^3/mL versus SR, 5.0 ± 1.6 × 10^3/mL; *P = NS*]. In contrast, CD34^+ levels remained elevated after CV(−) (n = 3) (Figure 1A). Baseline HPC levels did not predict the success of cardioversion.

CD34^+/CD117^+HPCs increased transiently 48 hours after cardioversion (0.19 ± 0.12 × 10^3/mL versus 48 hours, 0.59 ± 0.39 × 10^3/mL; *P < 0.01*) and declined to 0.16 ± 0.04 × 10^3 cells/mL during follow-up. CD34^+/CD117^+HPCs were not affected by the success of cardioversion.

Numbers of HPCs did not correlate with clinical variables such as age, AF duration, atrial size, left ventricular ejection fraction, or medical therapy (data not shown).

#### SDF-1α, VEGF, and ANP Levels/CelCellula

SDF-1α levels were highest during persistAF (2057.4 ± 514.9 versus SR, 1439.5 ± 267.4 pg/mL; *P < 0.01*). Patients with paroxAF had intermediate levels (1891.1 ± 341.8 versus SR,
VEGF levels were not significantly elevated in patients with persistAF (27.9±30.0 versus SR, 15.0±5.0 pg/mL; P<0.05). ParoxAF was not different compared with SR (1200.8±450.9 versus SR, 883.6±134.6 fmol/L; P=NS). ANP levels declined after CV(+) (2385.1±1497 versus 48 hours, 1468.2±508.2 fmol/mL; P<0.05) (Figure 1C). Especially in paroxAF, ANP levels correlated with SDF-1α (r=0.56; P<0.01) and CD34+ (r=0.76; P<0.01). Amounts of IL-6 and IL-10 were not elevated during persistAF (IL-6 <10 pg/mL; IL-10 <5 pg/mL; n=5). Cardioversion did not alter IL-6 and IL-10 levels. Cell cultures from patients with AF showed more MEF-2-positive cells (Δ85%; absolute 1.3%) and ANP-positive cells (Δ53%; absolute 2.3%) compared with SR (0.7% and 1.5%) (Figure 2). There was no difference in the number of CD31 (platelet endothelial cell–adhesion molecule-1 [PECAM1])–positive cells (AF: absolute 44% versus 42%; n=8). PECAM-1 serves as a marker for endothelial differentiation.
Discusison
To the best of our knowledge, this is the first study demonstrating increased CD34+HPCs in the blood of patients with persistAF. Interestingly, application of DC shocks temporarily reduces the amount of CD34+HPCs and increases CD34+/CD117+HPCs regardless of the underlying cardiac rhythm. In contrast, CD34+ levels were comparable in patients with paroxAF and SR.

Recent reports suggest that blood levels of CD34+HPCs and progenitor cells increase in response to cardiac injury.1,2 Chemokines such as SDF-1α and VEGF modulate the homing of HPCs in the bone marrow, and overexpression of these factors results in mobilization of hematopoietic cells in vivo, whereas no such relation was found for IL-3 and IL-6.1,8

AF triggers the release of ANP into the systemic circulation and causes degenerative atrial changes.4 In the present study, successful restoration of SR caused SDF-1α and ANP levels to return to normal, which was followed by a decrease in HPCs. ANP levels were related to SDF-1α and CD34+ levels. Interestingly, after 4 days of culture, more HPCs from patients with AF expressed ANP (Δ53%) and MEF-2 (Δ85%). Pathophysiologically, these findings imply that AF affects transdifferentiation of HPCs into cardiomyocytes, and thereby it may contribute to myocardial (atrial) tissue repair. Importantly, large amounts of ANP stored as amyloid are found in fibrillating atria.4 It is tempting to speculate that ANP contributes to increased amounts of MEF-2-positive cells in atrial tissue.2 This is supported by a recent study, which suggests a role of the oxytocin-ANP system in cardiomyogenesis.9 Further studies are needed, however, to prove ANP to be a homing factor for HPCs. Besides potential beneficial effects of HPC differentiation, recent findings suggest that progenitor cell–derived cardiomyocytes may have proarrhythmic effects. Thus, CD34+HPCs or specific subsets thereof may also contribute to AF or focal arrhythmias.3

In contrast to myocardial ischemia, CD34+/CD117+ cells are not increased during AF, and absolute cell counts are low, implying that they are of minor importance in this setting.1

In the present study, application of DC shocks caused a transient decrease of CD34+HPCs. It is tempting to speculate that described vascular effects of DC energy increase the adhesion/invasion of circulating HPCs to vascular endothelium, which can also explain the energy-dependent, rapid decline of circulating HPCs after cardioversion. In addition to these rapid alterations, endothelial damage is known to induce a slower humoral response resulting in increased VEGF levels.10 One previous study has reported increased VEGF levels in some patients with AF, suggesting that AF per se may affect VEGF.11 In our study, increased VEGF levels were related to the amount of CD34+/CD117+HPCs, explaining their increase 48 hours after cardioversion. However, endothelial differentiation of HPCs was not induced by AF.

In conclusion, AF and application of DC shocks have significant effects on blood HPCs. Further important studies are warranted to investigate the regulation of chemokine receptors such as CXCR4 during AF.

Acknowledgments
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References
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