Clinical and experimental evidence of prostaglandin E1-induced angiogenesis in the myocardium of patients with ischemic heart disease

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Abstract

Objective: Prostaglandin E1 (PGE-1) is a potent vasodilative agent which has been used to bridge patients with chronic heart failure listed for heart transplantation (HTX). In various experimental settings PGE-1 appears to stimulate angiogenesis by inducing vascular endothelial growth factor expression. This observational clinical study sought to investigate the angiogenic effects of PGE-1 in the failing human heart.

Methods: Neovascularization was investigated in 14 explanted hearts from patients with ischemic cardiomyopathy (ICMP) who had been bridged to HTX with PGE-1 (8\textpm1 mg/kg/min, 97\textpm75.6 days) and compared with 14 hearts who did not receive PGE-1 prior to HTX. In three sectional areas obtained from the left ventricular wall CD34, von Willebrand factor (vWF), nuclear Ki67 (MIB-1), and VEGF were quantified by immunohistochemistry to estimate capillary density and endothelial cell proliferation. Additionally, to investigate a possible angiogenic effect of PGE-1 in vitro, cultured human coronary artery smooth muscle cells (HCASMCs) were treated with PGE-1.

Results: PGE-1-treated patients had significantly more CD34- and vWF-positive cells in the subepicardium (both \(P<0.01\)), myocardium (both \(P<0.0001\)) and subendocardium (\(P<0.01\) and \(P<0.001\)) as compared to the non-PGE-1 group. Proliferative endothelial activity expressed by the presence of MIB-1- and VEGF-positive cells (both \(P<0.0001\) in all layers) was increased more than twofold. Addition of PGE-1 to HCASMCs in cell culture resulted in a significant increase in VEGF production (164.0\textpm19.7 pg/10\textsuperscript{5} cells/24 h, \(P<0.005\)) as compared to the control cell line (66.6\textpm8.7 pg/10\textsuperscript{5} cells/24 h, \(P<0.005\)).

Conclusions: Our data demonstrate that PGE-1 is a potent stimulator of angiogenesis via upregulation of VEGF expression. The induction of therapeutic angiogenesis in patients with severe ICMP might explain the favorable clinical outcome in PGE-1 treated patients until HTX.

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1. Introduction

Prostaglandin E1 (PGE-1) administered intravenously or intraarterially has been used for the treatment of peripheral occlusive artery disease (PAWD) as well as for end-stage heart failure in patients awaiting heart transplantation [1–4]. So far, the clinical effects of prostaglandins have been mainly attributed to their powerful vasodilative activity [5–7].

The use of PGE-1 in animal studies appeared to evoke stimulatory effects on angiogenesis in the cornea of rabbits as well as in the chorionallantoic membrane of chicken embryos [8,9]. Therefore, the mechanisms of the beneficial

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hemodynamic long-term effects and improved clinical situation in patients with severe ICMP might involve also neo-angiogenesis in the ischemic tissue.

Angiogenesis refers to the sprouting of capillaries and/or small non-muscular vessels from pre-existing capillaries, which is considered to be the predominant mechanism for increasing collateral blood flow in chronic myocardial ischemia. Accordingly, the expression of VEGF mRNA is temporally and spatially related to the development of capillaries [10]. Recent findings of an induction of vascular endothelial growth factor expression (VEGF) by PGE-1 in human monocytes [11] or in human synovial fibroblasts [12] implicate a more distinct role of PGE-1 in angiogenesis. Therefore, we hypothesized that exogenously administered PGE-1 might stimulate angiogenesis via VEGF expression in the failing human heart.

An observational study was undertaken using explanted myopathic hearts to estimate capillarisation, to assess specific angiogenic factors, e.g., VEGF, and to relate these findings to pretransplant treatment with or without PGE-1.

In order to quantify angiogenesis, we performed immunohistochemical staining for the endothelial cell markers CD 34 [13], von Willebrand factor (vWF) [14] and MIB-1 [15], reflecting Ki67-antigen, respectively. Presence of nuclear Ki67-antigen was considered helpful to indicate proliferative endothelial activity [16]. To elucidate the pathophysiological processes leading to PGE-1 mediated angiogenesis, we additionally determined the immunoreactivity against VEGF, a potent and specific mitogen for angiogenesis via VEGF expression in the failing human heart. We performed immunohistochemical staining for the endothelial cell markers CD 34 [13], von Willebrand factor (vWF) [14] and MIB-1 [15], reflecting Ki67-antigen, respectively. Presence of nuclear Ki67-antigen was considered helpful to indicate proliferative endothelial activity [16].

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Finally, to investigate a possible angiogenic effect of PGE-1 in vitro, cultured human coronary artery smooth muscle cells (HCASMCs) isolated from human coronary arteries of recipients’ hearts were treated with PGE-1 and VEGF production by these cells was determined.

2. Methods

2.1. Patients

Twenty-eight human hearts were obtained after orthotopic heart transplantation (HTX) from patients with endstage ischemic heart disease, as a result of ischemic cardiomyopathy (ICMP).

Prior to HTX 14 of these patients had received long-term i.v. PGE-1-infusions (Alprostadil), (PGE-1 group). The criteria for PGE-1 treatment were twofold: (a) patients were severely compromised in their daily activities, although they had received maximal oral triple therapy with ACE-inhibitors, diuretics and digitalis (NYHA class III/IV) and β-blockers (one patient). (b) Hemodynamic inclusion criteria were defined for cardiac index < 2.0 l/min/m² (CI) and for pulmonary capillary wedge pressure > 20 mmHg at baseline evaluation. As can be seen from Table 1 the hemodynamic parameters of the treated patients indicated a low cardiac index (CI < 2.0 l/min/m²), high pulmonary capillary wedge pressure (PCWP > 26 mmHg), high pulmonary artery mean pressure (PAMP > 38 mmHg) and a high pulmonary vascular resistance index (PVRI > 600 dyn·s·cm⁻5·m⁻²) (Table 1). The protocol under which the study was conducted was approved by the institutional ethics committee at the General Hospital of Vienna. The protocols of PGE-1 application were described previously in detail [1–4]. In brief, patients received stepwise increased PGE-1 infusions during right heart catheterization until the maximum tolerated dose (MTD) was reached, which was defined as the dose when side effects became intolerable (myalgia, arthralgia, hypotension, nausea, vomiting, diarrhoea, headache). After hemodynamic changes were documented, MTD (mean: 29 ± 1 ng/kg/min) was reduced by 50% for continuous infusion through the following 12 h. As soon as hemodynamic stabilization in connection with an acceptable tolerability of the drug was confirmed, patients were implanted a percutaneous central venous line (Hickman catheter), connected to a portable pump. Mean total time of PGE-1 was 50% for continuous infusion through the following 12 h. As soon as hemodynamic stabilization in connection with an acceptable tolerability of the drug was confirmed, patients were implanted a percutaneous central venous line (Hickman catheter), connected to a portable pump. Mean total time of PGE-1 was 50% for continuous infusion through the following 12 h. As soon as hemodynamic stabilization in connection with an acceptable tolerability of the drug was confirmed, patients were implanted a percutaneous central venous line (Hickman catheter), connected to a portable pump. Mean total time of PGE-1 was 50% for continuous infusion through the following 12 h. As soon as hemodynamic stabilization in connection with an acceptable tolerability of the drug was confirmed, patients were implanted a percutaneous central venous line (Hickman catheter), connected to a portable pump. Mean total time of PGE-1 was 50% for continuous infusion through the following 12 h.
Table 1
Clinical characteristics of patients at baseline

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Patient groups</th>
<th>PGE-1 group</th>
<th>Non-PGE-1 group</th>
<th>P value*</th>
</tr>
</thead>
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<tr>
<td>(1) General data</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>55.0±8.8</td>
<td>57.57±8.76</td>
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<tr>
<td>Sex (males/females)</td>
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<td>(13/1)</td>
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<td>NYHA</td>
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<td>3.68±0.31</td>
<td>3.23±0.46</td>
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<tr>
<td>(2) Oral medication</td>
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<tr>
<td>Furosemide (mg/day)</td>
<td></td>
<td>138.2±68.43</td>
<td>102.50±69.72</td>
<td>&lt;0.0001</td>
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<td>ACE-I, Enalapril (mg/day)</td>
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<td>33.2±8.68</td>
<td>32.86±19.88</td>
<td>n.s.</td>
</tr>
<tr>
<td>Digoxine (mg/day)</td>
<td></td>
<td>0.07–0.10</td>
<td>0.07–0.10</td>
<td>n.s.</td>
</tr>
<tr>
<td>(3) Cardiac rhythm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bradykardia</td>
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<td>0/0</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>(3) Hemodynamic baseline data§</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
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<td>26.93±7.63</td>
<td>23.79±3.68</td>
<td>n.s.</td>
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<tr>
<td>PAMP (mmHg)</td>
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<td>38.29±7.0</td>
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<td>n.s.</td>
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<td>CI (l/min/m²)</td>
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<td>2.39±0.26</td>
<td>&lt;0.001</td>
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<td>PVR (mmHg/l/min)</td>
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<td>4.44±0.92</td>
<td>2.66±0.41</td>
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<tr>
<td>PVRI (dyn×s×cm⁻³×m⁻²)</td>
<td></td>
<td>606.0±160.95</td>
<td>415.57±66.82</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

*Statistical difference between PGE-1 group vs. Non-PGE-1 group.

Hemodynamic baseline data were measured during right heart catheterization. Values are presented as mean±S.D.

Abbreviations: PAMP, pulmonary artery mean Pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; PVRI, pulmonary vascular resistance index indicates the effective pulmonary perfusion in relation to the cardiac output; CI, cardiac index; ACE-I, angiotensin converting enzyme inhibitor; SR, sinus rhythm; AFIB, atrial fibrillation; PM, pacemaker.

saline, pH 7.2). Tissue embedded in paraffin wax was cut in 3–5 μm thick sections, dried at 55 °C for 2 h and then deparaffinized in xylene for 20 min followed by dehydration through graded alcohols. The endogenous peroxidase activity was quenched with 3% H₂O₂ in methanol and afterwards tissue proteolysis was performed by pre-treatment with microwaves. Sections were then immersed in Tris-buffered saline (0.15 M NaCl, 0.05 M Tris–HCl, pH 7.6), and incubated with the following antibodies for 12 h at 4 °C at an appropriate dilution: (a) monoclonal mouse anti-CD34 antibody (Neomarker, Fremont, CA, USA), dilution 1:150; (b) polyclonal rabbit anti-vWF antibody (Neomarker, Fremont, CA, USA), dilution 1:50; (c) monoclonal mouse anti-MIB-1 antibody (Immunotech, Marseille, France), dilution 1:100; (d) polyclonal rabbit anti-VEGF antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), reacting with the 165, 189, and 121 amino acid splice variants of VEGF, dilution 1:500; (e) monoclonal mouse anti-HIF-1α antibody (Neomarker), dilution 1:50.

Anti-MIB-1 staining was considered positive only when localization was nuclear.

To correct for unspecific staining a polyclonal rabbit IgG and the respective mouse IgG were used as negative controls.

The detection of antibodies was performed with a Dako immunohistochemistry kit (Dako, Glostrup, Denmark). Three independent observers, who were blinded to the sample allocation, performed all investigations. The inter-observer variability was less than 2%.

2.3. Location of capillaries

The capillary density was determined separately in the subepicardium, the middle layer of the myocardium and the subendocardium [18] with one third of the transmural thickness attributed to each layer. On histological sections stained immunohistochemically with CD34, VEGF, vWF and MIB-1 the number of capillary profiles was counted in non-infarcted myocardium representing diffuse ischemic damage rather than a local ischemic event. At a magnification of 400×1, 25 visual fields were evaluated in each layer. The fields were chosen with systematic random sampling as described previously [4,15]. The results were presented as number of capillary profiles per square millimeter of cross sectional area.

2.4. Determination of fibrosis

Collagen fibers (collagen type III) were stained with sirius red F3 (Sigma, Vienna, Austria) [19]. We determined the fibrosis content of the myocardium with an automatic image analyzing system, using planimetric analysis (NIH Image 1.61/ppc; Bethesda, MD, USA) in a Zeiss Axiovert 135 TV microscope (Jena, Germany). In each sample, we evaluated the whole sectional area of the histologic section. The total areas ranged between 170 and 250 mm². The fibrosis grade (in % of total area) was calculated according to the equation (sirius red-positive area/total area)×100=% fibrosis.
2.5. Computer-assisted quantitative histological evaluation of HIF-1α in myocardial specimens

All specimens were digitized in their full size utilizing a slide scanner (Nikon 6.0 35 mm film scanner, LS-20, Nikon Corporation, Tokyo, Japan). Images were processed and the color contrast enhanced using the Adobe PhotoShop 3.0 software package (Adobe Systems, San Jose, CA, USA).

Planimetric analysis of the specimen area positively stained with anti-HIF-1α antibodies in percent (%) of total specimen area [immunoreactivity area or percentage (%) enrichment] was performed using computer-based planimetry (NIH Image 1.61/ppc) [20–22]. In addition, staining intensity was analysed using a score, ranging from 0 to 4 estimated units (eU) as follows: 0=no immunoreactivity, 1=minimal staining, 2=light staining, 3=intense staining and 4=very intense staining). As described in Section 2.2 we performed one transmural section from the middle part of the left ventricle in total per patient, containing the subepicardium, the middle layer of the myocardium and the subendocardium. The mean values of these determinations were used for statistical calculations. There was 100% reproducibility for the assessment of staining intensity, and a percentage difference between two observers (interobserver variability) of 1 eU or 20%.

2.6. Cell culture techniques

2.6.1. Isolation and characterization of smooth muscle cells

HCASMCs were isolated by the explant technique from pieces of human coronary arteries of recipients’ hearts obtained after heart transplantation [23]. These patients were not treated with PGE-1 prior to heart transplantation. The cells were confirmed to be smooth muscle cells (SMCs) by their typical “hill and valley” morphology and positive immunofluorescence staining with a monoclonal antibody against alpha SMC-actin (Boehringer Mannheim, Germany). Such SMCs were subcultured in M199 containing 10% supplemented calf serum; Hyclone, Logan, UT, USA) using a 1:3 split ratio and were used in passage 3 for the experiments described below. Human coronary artery endothelial cells (HCAECs) were isolated by mild collagenase treatment and characterized as described for macrovascular endothelial cells [24].

2.6.2. Treatment of cells with PGE-1

Cells were grown to confluence in six-well plates as described above. Twenty-four hours prior to the start of the experiment M199 containing 10% supplemented calf serum was replaced with serum free medium (M199 containing 0.1% bovine serum albumin). Thereafter the cells were rinsed and fresh serum free medium with and without PGE-1 at concentrations indicated in Table 2 was added. After 24 h of incubation the conditioned media of such treated cells was collected and stored at −70°C.

2.6.3. Determination of vascular endothelial growth factor (VEGF)

VEGF was determined in conditioned media of HCASMCs and HCAECs using a specific enzyme-linked immunosorbent assay (ELISA; Cytimmune Sciences Inc., College Park, MD, USA).

2.7. Statistical analysis

Values are presented as mean±S.D. All determinations of three independent observers were averaged and the mean value was included in the analysis. Analysis of variance (ANOVA) with the Scheffé procedure as a post-hoc test or the Student’s t-test was used to compare the means. A P value of <0.05 was considered to be significant.

3. Results

3.1. Patient characteristics

The patients of the PGE-1 group were of similar age and received comparable dosages of angiotensin converting enzyme inhibitors and loop diuretics compared to the patients of non-PGE-1 group (Table 1). As expected, in the patients of the PGE-1 group NYHA class turned out to be significantly higher (P<0.01 vs. P<0.01) accompanied by poor hemodynamic parameters at time of listing (Table 1). PGE-1 therapy lead to an improvement in all of these hemodynamic parameters, including CI (Fig. 1A), PCWP (Fig. 1B), PAMP (Fig. 1C), and PVRI (Fig. 1D) as compared to baseline values (Table 1). The P values were highly significant for all parameters (Fig. 1A–D, P<0.01 vs. prevale). Although the duration of PGE-1 therapy was inevitably very variable due to the limited availability of suitable donor organs, we did not observe any relationship


3.2. Immunohistochemical capillary markers

3.2.1. CD 34

CD 34 which has previously been shown to be expressed in endothelial cells in normal tissue and in benign and malignant proliferations was used as a specific marker of endothelial cells to validate microvessel density [13]. Using an antibody against this protein we found that the PGE-1 group had significantly more CD 34 positive capillaries (Fig. 2a and b) compared to the non-PGE-1 group (subepicardium: 1403±276 vs. 819±44 capillary profiles/mm², \(P<0.001\); myocardium: 1247±334 vs. 771.4±68.3/mm² capillary profiles/mm², \(P<0.0001\); subendocardium: 1296±359 vs. 805±39.1 capillary profiles/mm², \(P<0.01\)).

3.2.2. vWf

In order to control the results obtained with CD34 the expression of this second endothelial marker was investigated immunohistochemically [14]. After PGE-1-infusion therapy patients showed significantly more vWf positive capillaries (Fig. 2c and d) compared to patients not receiving PGE-1 (subepicardium: 1275±274.7 vs. 683±60.3 capillary profiles/mm², \(P<0.001\); myocardium: 1123±338 vs. 671±63.5 capillary profiles/mm², \(P<0.0001\); subendocardium: 1172±376.4 vs. 674±43.95 capillary profiles/mm², \(P<0.0001\)).

3.3. VEGF-immunostaining of capillaries

Indicating an angiogenic response, PGE-1-treated patients (Fig. 2e and 2f) showed significantly more VEGF-positive capillaries per mm² in all three sectional areas than patients not receiving PGE-1 (subepicardium: 608±127 vs. 247±46.8 capillary profiles/mm², \(P<0.0001\); myocardium: 554±125 vs. 238±53.6 capillary profiles/mm², \(P<0.0001\); subendocardium: 567±138 vs. 245±56.0 \(P<0.0001\)). In this respect, it has to be noted that myocytes as well as interstitial cells expressed VEGF to some extent, which is in accordance with previous findings [25].

3.4. MIB-1 (Ki 67 antigen)

The proliferative potential of the capillary endothelium was examined immunohistochemically using the monoclonal antibody MIB-1. Ki67 antigen is expressed throughout the cell cycle and reliably distinguishes proliferating from resting endothelial cells (Fig. 2g and h) [16]. In the PGE-1 group the number of proliferating capillaries was significantly higher (Fig. 1c) in all layers of the ventricular wall compared to non-PGE-1 (subepicardium: 13.3±2.4 vs. 6.6±2.8 capillary profiles/mm², \(P<0.0001\); myocardium: 9.9±2.4 vs. 5.0±2.56 capillary profiles/mm², \(P<0.0001\);
Fig. 2. Immunohistochemical detection of endothelial cell markers CD34 (A, B) and vWf (C, D) in a paraffin section of the myocardium in a representative PGE-1-treated patients vs. nonPGE-1 treated patient. Increased VEGF expression in myocardium and capillaries of PGE-1-treated patients (E) compared to nonPGE-1 patients (F). MIB-1-positive endothelial cells in myocardium, demonstrating proliferating activity of PGE-1-treated patients vs. nonPGE-1 group (G, H). Immunostaining for HIF-1α revealing a decrease of tissue hypoxia after PGE-1 treatment (I, J); light microscopic magnification 1:600 (panels A, B, C, D, E, F), 1:1200 (panels G, H), 1:400 (panels I, J).

subendocardium: 12.0±2.8 vs. 5.5±2.4 capillary profiles/mm², *P*<0.0001) (Fig. 2g and h). The data indicate a significant increase in proliferative activity.

3.5. Immunofluorescence double staining for CD34/MIB-1

To confirm proliferative activity of endothelial cells we
stained selected specimens of PGE-1 treated and non-treated ICMP patients simultaneously with phycoerythrin-labeled anti-CD34 and FITC-labeled-anti-MIB-1 antibodies. Representative pictures of the obtained results are given in Fig. 3.

3.6. Fraction of fibrosis

Although the hearts from PGE1-treated patients showed a reduction of collagen content after PGE1 treatment, the observed alterations did not reach any statistical significance as compared to non-treated controls (13.94±0.8 vs. 16.15±1.03%; P=0.11; Fig. 5).

3.7. HIF 1α-immunostaining

Hypoxia-inducible factor 1 alpha (HIF-1α), as a marker of tissue hypoxia, is considered to be an important transcriptional factor responsible for regulating expression of VEGF [17]. As can be seen from Fig. 2i and j and from Fig. 4, HIF-1α enrichment (in %) as well as staining intensity (in eU) is significantly decreased in the hearts of patients treated with PGE-1 as compared to non-treated controls (enrichment: 10.14±2.51 vs. 18.64±4.38%, P<0.01; staining intensity: 0.96±0.31 vs. 1.93±0.46 eU, P<0.01).

3.8. Cell culture

As can be seen from Table 2, PGE-1 increased VEGF expression by HCASMC at a concentration of $10^{-5}$ M significantly whereas PGE-1 at concentrations of $10^{-6}$ and $10^{-7}$ M had no effect. VEGF was not detectable in human coronary endothelial cells in the presence or absence of PGE-1 (Table 2).

4. Discussion

Several clinical investigations reported on the benefit of PGE-1 infusion as a bridge to HTX in patients with therapy refractory endstage ICMP [2,3,6–9] and an-
The angiogenic effect of PGE-1 in patients with idiopathic dilative cardiomyopathy has previously been demonstrated by our study group [4]. The results of the following study document an angiogenic response to PGE-1 treatment in hearts from patients with ICMP. We speculate that PGE-1 might play a crucial role in this process by inducing endothelial proliferation possibly via upregulation of VEGF expression. Our data demonstrated that PGE-1 treatment prior to HTX increased the capillary density, expressed by an increase in CD34- and number of VEGF-positive endothelial cells in non-infarcted myocardial areas in patients with ICMP when compared to hearts from ICMP-patients who did not receive PGE-1 bridging therapy.

The baseline hemodynamic parameters, especially cardiac index (CI), pulmonary capillary wedge pressure (PCWP), pulmonary artery mean pressure (PAMP), pulmonary vascular resistance (PVR), and pulmonary vascular
resistance index (PVRI) of the non PGE-1 group were better than those obtained in PGE-1 treated patients. Since the benefit of a PGE-1-infusion therapy in CHF-patients has been proved in previous studies [1–4], it would, however, not meet ethical demands to perform a placebo controlled, prospective study with control patients having the same identical hemodynamic outcomes as the PGE-1 patients.

In order to examine myocardial capillarisation immunohistochemical staining of endothelial cells was performed with antibodies against vWF and CD 34, which as a specific marker of endothelial cells is also expressed on stem cells of the hematopoietic system [26]. As the number of capillary profiles per sectional area is determined not only by the number and length of capillaries, but also depends on the sectioning angle [27], an accurate quantitation of the capillary network requires a three dimensional orientation of myocardial capillaries to be taken into account. Accordingly, measuring merely the capillary density, as the number of capillary profiles on cross sections, the true capillary supply of the myocardium may be overestimated due to the fact that maximum of capillary profiles is found in cross sections, but the minimum in longitudinal sections. By using random sections in our study, which are arbitrarily oriented in space, the mean density of capillary profiles equals the half of the mean length of capillaries per tissue volume (capillary length density). Thus, the capillary density observed in our study tends to be lower than densities from corresponding cross sections [27,28].

Considering the morphological analysis of the capillaries it is noteworthy to indicate a smaller fiber dimension in the PGE-1 treated group. Indeed, PGE-1 appears to reduce the degree of fibrosis to some degree, especially the collagen content in non-infarcted myocardial areas. Nevertheless, since the observed alterations in fiber dimension did not reach any statistical significance, the capillary to fiber ratio was finally not determined.

It has been shown by Mall et al., that chronic administration of the powerful vasodilating drug dipyridamol resulted in an increase in capillary growth in the rat heart mediated by mechanical shear stress after enhancement of myocardial blood flow [28]. While these findings of an increased number of capillaries could not necessarily be related to true capillary growth but might be explained by the opening of a previously nonperfused capillary reserve, a phenomenon commonly referred to as capillary recruitment, in our study the presence of nonperfused collapsed capillaries was precluded by electron microscopic examination of the myocardial interstitium (Fig. 5). Thus, our data cannot be explained in terms of capillary recruitment, but demonstrate true capillary growth.

Prostaglandins have been found to induce the expression of VEGF in various cells and tissues, including human monocytes [11] and human synovial fibroblasts [12]. Here we provide evidence that PGE-1 can induce the production of VEGF in human coronary artery smooth muscle cells in vitro whereas it had no effect on endothelial cells. Recent studies have identified smooth muscle cells as significant source of VEGF and have suggested a role for this cell type in the regulation of angiogenesis [29]. If this mechanism is also operative in the in vivo situation one could speculate that PGE-1 might induce VEGF expression in smooth muscle cells and possibly also fibroblasts and monocytes in the heart. Given the positive staining of cardiac myocytes and interstitial cells for VEGF and fibroblasts in our study a role for these cells in the regulation of angiogenesis in the in vivo setting must also be considered [23]. Such VEGF would then bind to its specific receptors on endothelial cells and induce angiogenesis. This would be in accordance with our results derived from patients with chronic ischemic heart disease, demonstrating that in heart specimens of PGE-1-treated patients the number of VEGF-positive capillaries is dramatically increased compared with untreated controls. Moreover, PGE-1-treated patients had significantly more CD34, vWF and MIB-1 positive cells in the subendocardium, myocardium and subepicardium. In addition, increased proliferation of endothelial cells in all layers of the ventricular wall was confirmed by double staining for CD34 and MIB-1 (nuclear Ki67 antigen), a strong indicator of enhanced proliferative activity [12,14,15].

Patients treated with PGE-1-infusions showed a remarkable improvement in the hemodynamic parameters as assessed by right heart catheterization [1,2]. This beneficial hemodynamic effect of PGE-1 is likely to be caused by various factors, including a vasodilative action resulting in a decline of pre- and afterload. Previously, it was demonstrated that angiogenesis in the ischemic heart leads to improved myocardial contractility [30]. However, the
observed amount of adaptive angiogenesis that is insufficient to restore normal tissue blood flow in vascular ischemic disorders including both ICMP and peripheral vascular disease (PVD) appears to be related to a failure of appropriate VEGF induction [25]. Concerning the molecular mechanism for this phenomenon, Levy demonstrated that hypoxic pre-treatment of rat cardiac myocytes resulted in the blunting of the ability of these cells to respond to subsequent hypoxia with an induction of VEGF due to a failure of VEGF transcription [31].

Since the PGE-1 treated group was generally sicker, as documented by the hemodynamic baseline data, one might assume that the potentially increased level of cardiac ischemia with a resultant stimulation of HIF-1α-VEGF expression in these patients could be the source of angiogenesis rather than PGE-1 treatment per se. However, to ensure the level of tissue hypoxia, immunohistochemical staining of HIF-1α revealed lower protein levels in the PGE-1 treated group than in non-treated ischemic controls. Previously, it was demonstrated that prostaglandins are capable of stimulating angiogenesis in various tissues [8,9,11,12]. Furthermore, it is known from previous studies that hypoxia or chronic ischemia is a recognized stimulus of transcriptional HIF-1α activation [17]. In our study neoangiogenesis was accompanied by a significant decrease in HIF-1α. One could speculate that PGE-1 caused a sufficient increase in tissue oxygenation in patients with ischemic cardiomyopathy, finally leading to an improved ventricular contractility. Since we have no data to analyze the kinetics of angiogenesis in response to hypoxia we can not rule out completely the possibility that angiogenesis was originally triggered by the more severe disease in the treated patients. The reduction of HIF-1α, however, lends support to the notion that hypoxia is not the cause for ongoing VEGF production and subsequent neovascularization. In addition, we can not rule out that PGE-1— as described above— besides its direct effect on VEGF production in smooth muscle cells might induce other growth factors and cytokines, which then in turn could upregulate VEGF expression in a paracrine way thereby contributing to stronger angiogenesis despite lower HIF-1α expression in PGE-1-treated patients.

In conclusion, chronic PGE-1 infusion, which is a well-known and reliable therapeutic option in the treatment of ICMP has shown to be beneficial in terms of improvement of the clinical stage and symptoms. The controlled expression of angiogenic factors combined with the improvement of hemodynamic parameters in patients, refractory to conventional or interventional therapeutic strategies offers a very interesting and efficient therapeutic alternative based on induction of angiogenesis.

Acknowledgements

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References

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