Preclinical research

Attenuation of cardiac remodelling by endocardial injection of erythropoietin: ultrasonic strain-rate imaging in a model of hibernating myocardium

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Aims The aim of this study was to investigate whether erythropoietin (EPO) has cardioprotective effects in a chronic myocardial ischaemia model regarding strain-rate imaging parameters during dobutamine stress echocardiography (DSE).

Methods and results An ameroid constrictor was placed around the circumflex artery in 13 pigs to induce hibernating myocardium by a chronic vessel occlusion. The pigs were randomized 14 days later: seven pigs receiving 10 000 U EPO and six pigs receiving placebo injected into the ischaemic region using a NOGA™-guided transendocardial catheter. At weeks 2 and 6, animals were examined by DSE, electromechanical mapping, and coronary angiography. During incremental dobutamine infusion, regional radial function was monitored by measuring peak systolic strain-rates (SRsys), systolic strains (e1sys), and post-systolic strains (e1ps). At week 6, the animals were pathohistologically investigated. Echocardiography revealed 2.2 ± 0.8 hypokinetic segments in the EPO-treated animals in comparison with 3.3 ± 0.9 akinetic segments per animal in the controls. The mean ejection fraction was reduced in the control group (55 ± 3 vs. 66 ± 4%, P = 0.057). Strain-rate imaging revealed ischaemic myocardium in EPO-treated animals and non-viable myocardium in the controls (P = 0.0001). Histological analysis of the ischaemic region revealed a reduction of myocardial fibrosis (8 ± 1 vs. 27 ± 5%) in the EPO-treated group. The transmural extension of fibrosis and the echocardiographic deformation data correlated in the posterior walls (EPO group): e1sys at rest r = 0.83; peak SRsys during dobutamine stimulation r = 0.92, P = 0.01.

Conclusion Endocardial EPO injection may induce cardioprotective effects in chronic ischaemic myocardium and helps to obtain the myocardial contractile reserve, objectified by ultrasonic strain-rate imaging.

KEYWORDS Dobutamine stress echocardiography; Strain-rate imaging; Myocardial viability; Erythropoietin

Introduction

The therapeutic effects of erythropoietin (EPO) have been established for patients with renal anaemia since the mid-1980s.¹ Besides the promoting effects on erythropoiesis, first clinical safety studies revealed neuroprotective effects of EPO in patients with acute cerebral ischaemia.² Owing to the assessment of EPO receptors in myocardial muscle and fibrotic cells, EPO has been recently investigated in acute myocardial infarction models.³,⁴ Anti-apoptotic effects of EPO as well as a stimulated differentiation of myoblasts, mobilization of endothelial progenitor cells, and an increased angiogenic activity in myocardial endothelial cells have been described.⁵–¹⁰ Although cardioprotective effects of EPO in acute myocardial infarction could be assessed previously, the intention of this study was to investigate whether the effect of EPO can prohibit cardiac remodelling in chronic ischaemic myocardium.¹¹,¹² Because ultrasonic deformation parameters can define the transmural extension of the myocardial damage after infarction, the present study was performed to objectify and compare the cardioprotective effects of EPO application with histopathological findings.¹³

Methods

Experimental protocol

An ameroid constrictor was placed around the circumflex artery (CX) during off-pump thoracic surgery under anaesthesia and mechanical ventilation in a series of domestic pigs. After 2 and 6 weeks coronary angiography, stress echocardiography and electroanatomical mappings were performed under intravenous propofol 2% and fentanyl...
anaesthesia. The animals in the study were randomized into two groups: at week 2, seven pigs received 10 000 U EPO via endocardial injection and six pigs serving as controls received placebo injections. The volume of each injection was 100 μL. In addition, laboratory analysis of haemoglobin was performed at weeks 2 and 6, to investigate any stimulating effects of EPO on the haematopoiesis. After 6 weeks, all animals were sacrificed and the hearts were excised for histopathology (Figure 1).

Animal instrumentation
Seventeen domestic pigs, weighing 28–34 kg each, were housed and studied according to the guidelines of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The study was approved by the local Bioethical Committee in Kiel, Germany. Four pigs died before evaluation due to non-procedure-related infectious diseases (septicaemia).

Coronary angiography, electromechanical mapping, and endocardial injection
After biplane coronary and left ventricular angiography, a mapping catheter was placed via the aortic valve into the left ventricle. The endocardial mapping technology using the NOGA system has been previously described. Under fluoroscopic and echocardiographic guidance, 68 ± 20 points were acquired for 3-D reconstruction of the total left ventricular inner surface on the basis of multiple, non-overlapping triangular segments. According to a nine-segment model (four basal segments, four midventricular segments, one apical segment), the regional unipolar voltage and the local linear shortening were assessed. The analysis of the unipolar voltage served as an additional tool for the identification of viable (ischaemic) and non-viable myocardium, and the local linear shortening as a parameter of the regional contractility.

At week 2, after completing the left ventricular mapping procedure, all pigs received 10 endocardial injections of 1000 U EPO volume each or placebo using the MyoStar injection catheter. The area at risk was defined according to the unipolar voltage values and the echocardiographic findings. At week 6, after the second angiography and mapping procedure, the chronic ischaemic area was marked with ink using the injection catheter MyoStar so that the tissue specimen could be identified precisely after sacrifice of the animals.

Echocardiographic data acquisition
Two experienced cardiologists, blinded to the injection group, assessed regional contractility and deformation properties of chronic ischaemic and non-ischaemic myocardium both at rest and during an incremental dobutamine infusion (2.5, 5, 10, 20, and 40 μg/kg/min) at week 2 (time of injection) and week 6 after ameroid constritor implantation. Transthoracic echocardiography was performed with a Vivid 7 (GE Healthcare, 5-MHz transducer). All data sets were acquired during a brief apnoea over three consecutive heart cycles. Transmural radial strain-rates and strain were calculated from tissue velocities. Care was taken to align the Doppler beams with the vector of motion of the investigated wall. A 3–5 mm sample length was used for strain-rate calculation. A septal myocardial velocity profile was used to time end diastole and end systole. Data were acquired at 140–220 frames per second using previously described methodology. The echocardiographic investigations were performed at week 2 (time of injection) and week 6 after ameroid constritor implantation.

Echocardiographic data analysis
Standard grey-scale M-mode images were acquired for analysis of myocardial end-diastolic and end-systolic wall thickness at rest and during dobutamine stimulation. Colour Doppler myocardial imaging data were acquired with dedicated software (TVI, GE Healthcare). Regional radial deformation was quantified in basal and midventricular short-axis views for posterior segments (area of interest) and septal segments (remote region) by strain-rate imaging. From the averaged strain-rate and strain data, peak systolic strain-rate (SRsys), systolic strain (fsys), and post-systolic strain (fpost) were calculated (Figure 2). Peak fsys was defined as the magnitude of deformation from end diastole to end systole. Post-systolic strain was measured from end systole to time of maximum diastolic strain. In addition, left ventricular volumes and ejection fraction were determined by biplanar Simpson’s method in the two- and four-chamber views.

Material
EPO (Jansen-Cilag, Germany; OrthoBiotech, NJ, USA); placebo solution: NaCl 74.90 mM, NaH2PO4 8 mM, Na2PO4 11.27 mM, Glycine 66.67 mM, Tween 80 0.03%.

Histopathological studies
At week 6, the hearts were excised and fixed with 4% phosphate-buffered formalin. Heart tissue from the ink-marked area at risk and the remote septal area were embedded in paraffin, sectioned and stained with elastica-van-Gieson, Masson’s trichrome, and haematoxylin/eosin. Light microscopy was performed with an IX 71 Olympus microscope (Olympus, Germany). Morphometric quantification of fibrosis was performed by use of the analysis digital soft imaging system (version 3.2). Fibrosis was analysed in five bi-colour-coded views in each of the five samples of the Masson’s Trichrome-stained specimen. By planimetry the degree of fibrosis was expressed as percentage of myocardial tissue per field of view (2.38 mm²) by Masson’s trichrome and elastica-van-Gieson staining.

Immunohistochemistry
For immunohistochemistry, the APAAP method was used. Primary antibodies bound with a secondary antibody coupled with an enzyme-anti-enzyme-complex. After Fast-Red colouring, we performed nuclear staining with Wright-Giemsa. Microvessel density (number × 100) and vessel area (μm²) in the area at risk and in the remote septal area were analysed in α-SMA-smooth muscle
cell antigen; Santa Cruz Biotechnology, USA, 1:250) stained slices in five high power fields in each of five slices per region.

TUNEL assay
Apoptosis assessment was performed applying the terminal deoxy-nucleotidyl transferase-mediated nick-end labelling (TUNEL) assay according to the manufacturer’s instructions (in situ cell death detection kit, Roche Applied Science). Slices were screened in high power fields (400×). Breast cancer probes showing a high degree of apoptosis served as positive controls.

Adrenergic receptor analysis
Total β-adrenergic receptor (BRD) density was determined with the hydrophilic, non-selective radioligand 3H-CGP 12177A (Amersham Biosciences). Non-specific binding was determined in the presence of cold CGP 12177A 10 μmol/L. In an initial saturation experiment, the Kd was determined at 0.87 nM (data not shown). BRD density in samples was subsequently determined at a radioligand concentration of 2.5 nM.

Statistical analysis
Data are presented as mean ± standard error. Comparisons of the segmental data between the different groups (EPO vs. controls) were performed by use of variance analysis (general linear modelling). In the initial calculation, the treatment groups were defined as independent between-group factor. No covariates were included in the analysis. Univariate significances were analysed by the Greenhouse-Geisser test. The relative ischaemic area of EPO-treated pigs and controls as well as the ejection fraction were compared using independent samples t-tests controlled for equality of variances. A linear Pearson correlation was used to compare regional deformation and the extent of fibrosis. The data were analysed exploratory, i.e. P-values were computed two-tailed and no adjustment for multiple tests was made. Statistical significance was accepted for a value of P < 0.05.

Statistical analysis was performed using SPSS for windows 11.5.2.1, SPSS Inc.

Results
In all 13 pigs, either post-mortem myocardial staining or histology confirmed that the posterior myocardium had been included in the chronic ischaemic region as area at risk. Morphometric analysis revealed a significantly lower degree of fibrosis in the EPO group in comparison with the control group (8±1 vs. 27±5%, P = 0.01) 6 weeks after amiodarone constrictor implantation (Figures 3 and 4). The area of patchy and interstitial fibrosis extended through more than half of the myocardial depth in four of six pigs.
in the control group and in one of seven pigs in the EPO group (67 vs. 14%). In the non-ischaemic septal region, histology was normal in all animals. Moreover, in all 13 animals, the endocardial ink injections in the final experiments were located in the posterior myocardial segments assessed by histopathological analysis.

**Coronary angiography**

Standard biplane coronary angiography at weeks 2 and 6 revealed total occlusion of the proximal CX in all animals without any collateralization. Left ventricular function was observed to be reduced in controls at week 6, but did not reach statistical significance in comparison with the EPO-treated group (54 ± 3 vs. 63 ± 4%).

**Electromechanical mapping**

Details of the electromechanical mapping approach of this study have been reported previously. Endocardial electromechanical mapping showed an increase of the mean unipolar voltage amplitude in the ischaemic myocardial region in the EPO-treated group from 8.5 ± 0.4 mV at week 2 to 10.6 ± 1.5 mV at week 6 and a decrease from 8.9 ± 0.4 to 8.3 ± 0.8 mV in the control group. Both groups showed significantly reduced unipolar voltage in comparison with remote regions (septal) ($P = 0.0006$). While the mean count of ischaemic segments decreased in the EPO group from 3.6 ± 0.4 at week 2 to 2.7 ± 0.4 at week 6, the segment number of the controls increased from mean 3.0 ± 0.4 to 5.5 ± 0.6 segments ($P = 0.002$). In EPO-treated animals, the ischaemic surface area was significantly reduced in comparison with the control group at week 6 (19 ± 4 vs. 41 ± 2%, $P = 0.0007$) (Figures 5 and 6).

**Echocardiographic data**

2-D imaging and M-mode measurements

The mean ejection fraction remained nearly unchanged in the EPO group before and after treatment (64 ± 3% before, 66 ± 4% post). In the control group, the ejection fraction decreased from 60 ± 5% before to 55 ± 3% during follow-up. The final mean ejection fraction was higher in the EPO-treated group in comparison with controls, but did not reach statistical significance ($P = 0.057$). The treated group showed 2.8 ± 0.8 hypokinetic segments per animal before and 2.2 ± 0.8 hypokinetic segments after EPO injection, whereas 2.5 ± 0.7 abnormal segments before and 3.3 ± 0.9 akinetic segments after placebo injection could be detected in the control group. There were no significant differences in left ventricular end-diastolic cavity size between the treated and untreated groups at week 6 (40 ± 1 vs. 42 ± 2 mm, $P = 0.32$).

![Box plots of the percentage of the myocardial fibrosis in treated pigs (EPO group) and in the control group (placebo). The bold line shows the median value.](image)

**Figure 4**

![NOGA™ inner surface maps of the left ventricle based on the unipolar voltage at week 6. Post-injection map of (A) an EPO-treated pig, (B) an animal of the control group. The red zone represents the area at risk after occlusion of the circumflex artery post-injection at week 6.](image)

**Figure 5**
At week 2, a typically biphasic response of the regional wall motion was seen in all animals under dobutamine stimulation in the posterior segments. Initial improvement under low-dose dobutamine stimulation due to the obtained contractile reserve of viable myocardium was followed by further regional worsening (akinesis) under higher stimulation (20 \( \mu \)g/kg/min) as a sign of myocardial ischaemia.

End-diastolic and end-systolic wall thickness of the remote region (septal) did not show significant changes at rest at week 2 in comparison with week 6. While end-systolic posterior wall diameters did not differ significantly between the EPO and the control group at week 6 (10.8 ± 1 vs. 9.5 ± 1 mm, \( P = 0.52 \)), systolic wall thickness increased significantly in the EPO group during low-dose dobutamine stimulation (10 \( \mu \)g/kg/min) in comparison with the non-treated animals (14.8 ± 1 vs. 10.5 ± 1 mm, \( P = 0.02 \)).

While akinetic segments of the controls remained unchanged during dobutamine stimulation at week 6, the EPO-treated animals showed a typical biphasic response during dobutamine stimulation regarding the regional wall motion of the posterior segments and, additionally, post-systolic thickening in each animal.

Deformation in the EPO group

At week 6 at rest, \( S_{\text{sys}} \), \( e_{\text{sys}} \), and \( e_{\text{ps}} \) in the posterior wall averaged 3.6 ± 0.1 s \(^{-1} \), 36 ± 1%, and 9 ± 1%, respectively. During dobutamine stimulation, a biphasic \( S_{\text{sys}} \) response with an initial increase with a maximum at 10 \( \mu \)g/kg/min (5.4 ± 0.2 s \(^{-1} \)) was followed by a further decrease under continued dobutamine stimulation (Figure 7). While \( e_{\text{sys}} \) remained at the level of about 36 ± 1% during the incremental dobutamine stimulation, \( e_{\text{ps}} \) increased to 27 ± 2% at 40 \( \mu \)g/kg/min. During dobutamine infusion, the systolic thickening of the posterior wall decreased incrementally, whereas post-systolic thickening increased progressively (Figure 8). At 40 \( \mu \)g/kg/min, most myocardial thickening occurred after end systole. In the EPO-treated animals, there was a significant correlation between the transmural extension of fibrosis and the echocardiographic deformation data in the posterior walls: \( e_{\text{sys}} \) at rest at week 6 (\( r^2 = 0.83 \), \( P = 0.01 \)); peak \( S_{\text{sys}} \) during dobutamine stimulation at week 6 (\( r^2 = 0.92 \), \( P = 0.01 \)).

At week 2, an initial increase of \( S_{\text{sys}} \) was followed by a further decrease under continued dobutamine injection. During the incremental dobutamine stimulation, \( e_{\text{ps}} \) increased to 28 ± 2% at 40 \( \mu \)g/kg/min.

Deformation in the control group

At week 6 at rest, there was almost no deformation in the infarcted posterior wall; \( S_{\text{sys}} \), \( e_{\text{sys}} \), and \( e_{\text{ps}} \) averaged 2.0 ± 0.1 s \(^{-1} \), 4.8 ± 0.8%, and 4.0 ± 0.4%, respectively. During dobutamine stimulation, deformation parameters did not change in the infarct zone (Figure 7). For each dobutamine stage, \( S_{\text{sys}} \), \( e_{\text{sys}} \), and \( e_{\text{ps}} \) were significantly lower (\( P = 0.0001 \)) than in the EPO group. Moreover, there was a significant correlation between the transmural extension of fibrosis and \( e_{\text{sys}} \) at rest at week 6 in the posterior wall in the controls (\( r^2 = 0.86 \), \( P = 0.028 \)). Weaker correlations were found between the extension of fibrosis and peak \( S_{\text{sys}} \) during dobutamine stimulation (\( r^2 = 0.83 \), \( P = 0.01 \)).

At week 2, the deformation data of the EPO group and the controls were similar at week 2 before the EPO injection and did not differ significantly (Figure 7).

Deformation in the remote region

In the EPO-treated animals at rest at week 6, \( S_{\text{sys}} \), \( e_{\text{sys}} \), and \( e_{\text{ps}} \) in the septal wall averaged 3.2 ± 0.1 s \(^{-1} \), 33 ± 1.5%, and 3 ± 0.4%, respectively. During dobutamine stimulation, \( S_{\text{sys}} \) increased nearly linearly to 16.5 ± 0.9 s \(^{-1} \) at 40 \( \mu \)g/kg/min. The septal \( e_{\text{sys}} \) showed a biphasic response, with highest values at 10 \( \mu \)g/kg/min (58 ± 1.9%) and subsequent decrease under further stimulation. There was almost no...
$e_{ps}$ in the normal perfused septum either at rest or during dobutamine stimulation (unchanged 3 ± 0.4%). In comparison with the deformation data of week 2, no significant changes could be assessed in the remote region, neither at rest nor during dobutamine stimulation.

In the control group, deformation data at rest and under dobutamine stimulation were similar and did not differ significantly from the EPO group (Table 1).

**Microvessel analysis**

The alpha-SMA microvessel count revealed 9.1 ± 0.8 high power field (EPO group) vs. 10.3 ± 1.1 (control group) in the areas at risk without a significant difference, whereas in both groups the normal (remote septal area) myocardial segments showed lower counts (6.8 ± 0.8 and 7.2 ± 0.8). No difference was seen between the EPO and control groups.
group in the vessel area measured by morphometry (5134 \( \mu \text{m}^2 \times 100 \) vs. 5187 \( \mu \text{m}^2 \times 100 \)) (Figure 9).

TUNEL assay

In the area at risk, positive cells were rarely detected in the TUNEL assay (<0.001%). Those cells were not myocardial cells and were seen outside of the myocardial cell structure (Figure 10). There was no significant difference between the EPO and the control group. Breast cancer probes serving as positive controls showed more than 50% TUNEL-positive cancer cells.

Adrenergic receptor analysis

No significant effect of ischaemia and/or EPO-treatment on \( \beta \)-adrenergic density was detected (\( P = 0.64 \)).

Laboratory findings

Haemoglobin values were similar in both groups over time and did not differ significantly between the groups. In both groups haemoglobin increased from week 2 (EPO group: 8.9 ± 0.3 g/dL; placebo group 7.9 ± 0.3 g/dL) to week 6 (EPO group: 9.7 ± 0.4 g/dL; placebo group 9.2 ± 0.4 g/dL) corresponding to the normal values in growing pigs.

Discussion

Cardioprotective effects of EPO in acute or chronic myocardial ischaemia models were described in previous studies.\(^3\),\(^11\),\(^12\) On the basis of its anti-apoptotic effects, a reduction of infarct size and an enhanced cardiac function were observed in a coronary vessel ligation model.\(^12\) In contrast to previous studies, a porcine chronic ischaemic model was used by implantation of an ameroid constrictor. Hibernating myocardium was induced by gradually occluding the CX within 14 days, followed by endocardial injection of EPO or placebo in the region of ischaemia. At this time, dobutamine stress echocardiography (DSE) and strain-rate imaging assessed ischaemic and viable myocardium with a typical biphasic response.\(^18\),\(^19\) This is important, because anti-apoptotic effects of EPO have to be supposed only in viable myocardium, but not in scar tissue.\(^5\) One major finding of this study was that the degree of fibrosis in the area at risk was significantly reduced in EPO-treated pigs correlating to the ultrasonic strain-rate imaging data.

Assessment of local deformation by strain-rate imaging

The amount of fibrosis correlated strongly with local deformation indices \( SR_{sys} \) and \( \varepsilon_{sys} \) at rest. According to the

![Figure 8](image-url) Radial strain profiles over one cardiac cycle in an EPO- and a placebo-treated pig during dobutamine stimulation, derived at week 6 from posterior (green curve) and septal wall (yellow curve). (A) EPO-treated pig: septal strain with normal profile (peak systolic 30%); thickening (strain) of the posterior wall is reduced during systole and continues during early diastole (post-systolic thickening), resulting in a delayed thickening peak. (B) Placebo-treated pig: normal septal strain (peak systolic 26%); there is no systolic thickening of the posterior wall corresponding to a transmural infarction.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Regional functional deformation parameters during low- and high-dose dobutamine stimulation of EPO-treated animals and the control group at weeks 2 and 6 in the remote region (septal wall)</th>
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<tbody>
<tr>
<td></td>
<td>EPO group (( n = 7 ))</td>
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<tr>
<td></td>
<td>Rest</td>
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<tr>
<td>Week 2</td>
<td></td>
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<tr>
<td>HR</td>
<td>65 ± 3.5</td>
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<tr>
<td>( SR_{sys} )</td>
<td>3.0 ± 0.2</td>
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<tr>
<td>( \varepsilon_{sys} )</td>
<td>34 ± 2.6</td>
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<tr>
<td>( \varepsilon_{ps} )</td>
<td>3.2 ± 0.4</td>
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<tr>
<td>Week 6</td>
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<tr>
<td>HR</td>
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<tr>
<td>( SR_{sys} )</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>( \varepsilon_{sys} )</td>
<td>33 ± 1.5</td>
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<tr>
<td>( \varepsilon_{ps} )</td>
<td>3.4 ± 0.4</td>
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findings of Weidemann et al.,\textsuperscript{13} who previously reported the diagnostic value of strain-rate imaging for defining of the transmurality of a chronic myocardial infarction, regional systolic deformation was significantly reduced at rest, especially in non-treated animals. Thus, the lower the systolic deformation, the greater the regional transmural extension of the scar. In EPO-treated animals, dobutamine stimulation induced a biphasic response at week 6 regarding regional wall motion as well as $SR_{sys}$ as a sign of myocardial viability. In contrast to the control group, the phenomenon of dose-dependent increase in post-systolic thickening was only observed in the EPO group at week 6. The presence of post-systolic thickening would again suggest that this may be a marker of segmental ischaemia and myocardial viability.\textsuperscript{20,21} In EPO-treated animals, regional strain parameters at rest and during dobutamine stimulation were similar to those of chronic non-transmural myocardial infarctions.\textsuperscript{13} In the control group, the scar distribution was characterized

![Figure 9](image-url) Data of microvessel analysis in the area at risk and in normal segments in treated pigs (EPO group) and in the control group. (A) Count of microvessels. (B) Area of microvessels.

![Figure 10](image-url) TUNEL assay of ischaemic myocardial region (400×). Red stained TUNEL positive nucleus of a non-myocardial cell, which is outside of the myocardial cell structure. TUNEL positive (apoptotic) cells were seen rarely as described in the Discussion section.
by no measurable systolic deformation or thickening either at rest or during dobutamine stimulation, corresponding to transmural infarcted myocardium.\textsuperscript{22}

Regional wall motion and systolic left ventricular function

Although the reduction of global systolic myocardial function did not reach statistical significance between treated and non-treated animals (ejection fraction $66 \pm 4$ vs. $55 \pm 3\%$, $P = 0.057$, respectively), regional deformation properties differed significantly. In contrast to EPO-treated animals, the number of segments with regional wall motion abnormalities increased significantly in the control group indicating an active process of a chronic ischaemia and hibernation.\textsuperscript{23}

Electromechanical mapping

In addition, endocardial electromechanical mapping revealed an increase of unipolar voltage in the ischaemic segments and a significantly reduced ischaemic surface area in EPO-treated animals compared with the control group suggesting a decline in ischaemic injury.\textsuperscript{15,24}

Moreover, in our study, EPO was directly applied into the chronic ischaemic region via an endocardial injection catheter. In contrast to a systemic application of EPO, precise injection implies a higher localized intramyocardial concentration of EPO.\textsuperscript{25} Histopathology revealed the correct identification of the region of chronic ischaemia by the analysis of the ink-marked myocardium in each animal.

Microvessel analysis

During vessel maturation endothelial tubes are formed. Without supporting smooth muscle cells, vessels are unstable and unorganized. Their function concerning myocardial perfusion is doubtful.\textsuperscript{26,27} Therefore, we investigated mature microvessel density and vessel area by applying smooth muscle cell antibodies for detection of angiogenesis. In contrast to in vitro studies\textsuperscript{7} showing angiogenic effects of EPO, our study did not reveal that cardioprotective effects of EPO are associated with angiogenesis. In both groups (EPO and control group) angiogenesis was equally increased in the area at risk compared with normal myocardium.

Clinical implications

The differentiation of transmural and non-transmural infarction as well as the identification of viable myocardium in chronic ischaemia has important therapeutic and prognostic implications.\textsuperscript{28} This study demonstrated that EPO administered by endocardial injection has cardioprotective effects in chronic ischaemic myocardium by the reduction of the transmural extension of the scar.

Anti-apoptosis

Since cardioprotective effects of EPO are based on anti-apoptosis, we searched for apoptotic myocardial cells in the specimen of the area at risk. However, relevant numbers of apoptotic cells could not be detected, but we cannot exclude that apoptosis rates might have been higher at earlier time points, as we only examined the heart specimen at week 6 after constrictor implantation. Apoptosis rates are known to be high in early stages of acute myocardial infarction\textsuperscript{29} and lower in later stages (0.1%). The extent of apoptosis in hibernating myocardium is controversial and the rates of apoptosis are highly different between studies.\textsuperscript{30} Besides methodological differences (specificity of the TUNEL assay, sensitivity of electron microscopy) apoptosis rates are high after short-term hibernation.\textsuperscript{31} In the ischaemic model of hibernating myocardium used here, myocardial apoptosis in the EPO and the control group was rare at week 6 (<0.001%). Therefore, cardioprotection of EPO in hibernating myocardium by anti-apoptosis remains to be proved.

Strain-rate imaging

By use of ultrasonic strain-rate imaging, ischaemic and viable myocardium can be identified quantitatively by investigation of the local deformation with high spatial and temporal resolution.\textsuperscript{13,18,32–34} Transmural and non-transmural infarcted myocardium can be differentiated with high diagnostic accuracy. Changes in $S_{Rs}$ correlate with global contractility and are relatively heart-rate independent.\textsuperscript{35,36}

Limitations

In this study, baseline investigations like coronary angiography, echocardiography, and electromechanical mapping were primarily performed at week 2, before randomization in treated and non-treated animals. Before implantation of the constrictor device at week 0, no baseline investigations were performed. Regarding the assessment of myocardial viability, no correlative PET data could be acquired before EPO treatment and during follow-up. The identification of the area at risk (ischaemic, but viable myocardium) was based on echo data.

A potential limitation is the small number of investigated animals. Four pigs died before evaluation due to non-procedure-related infectious disease and were excluded from the study.

Intramyocardial applied EPO offers theoretically additional benefits in comparison with the systemic application caused by higher localized concentration of EPO in the region of interest, but has to be proved in further comparative experimental studies.

In this study, EPO was applied 2 weeks after ameroid constrictor implantation in a model of chronic myocardial ischaemia. Using ultrasonic strain-rate imaging, viable but chronic ischaemic myocardium could be assessed. However, a relevant number of apoptotic cells could not be detected. The best time point of injection as well as the most effective dose of EPO has to be investigated in further studies. It is possible that the endomyocardial application of EPO closer to the time point of the coronary artery occlusion has even more cardioprotective effects as already seen at week 2.

Altered adrenergic receptor density with increase of $\alpha$-adrenergic receptors and decrease of BRD correlates with worsening inotropic reserve.\textsuperscript{37} In our study, no significant effect of ischaemia and/or EPO-treatment on $\beta$-adrenergic density could be detected. However, due to the limited sample size and interindividual variability, an effect cannot definitely be excluded.
Conclusions

Percutaneous endocardial injection of EPO in chronic, ischemic myocardium can reduce the infarct size and obtain regional myocardial viability. By using ultrasonic strain-rate imaging, the area at risk after vessel occlusion can be detected and cardioprotective effects of EPO can be objectified by quantitative analysis of the regional deformation properties at rest and during dobutamine stimulation.

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Conflict of interest: none declared.

References


