Effects of intracoronary delivery of allogenic bone marrow-derived stem cells expressing heme oxygenase-1 on myocardial reperfusion injury

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Summary
Heme oxygenase-1 (HO-1) decreases apoptosis, inflammation and oxidative stress. The aim of the study was to investigate the effects of intracoronary infusion of allogenic bone marrow cells (BMC) overexpressing HO-1 in the porcine model of myocardial infarction (MI). MI was produced by balloon occlusion of a coronary artery. BMC were transduced with adenoviruses encoding for HO-1 (HO-1 BMC) or GFP (GFP-BMC) genes. Prior to reperfusion animals received HO-1 BMC, control BMC (unmodified or GFP-BMC) or placebo. Left ventricular (LV) ejection fraction (EF), shortening fraction (SF), end-systolic and end-diastolic diameters (EDD, ESD) were assessed by echocardiography before, 30 minutes (min) and 14 days after reperfusion. BMC significantly improved LVEF and SF early (30 min) after reperfusion as well as after 14 days. Early after reperfusion HO-1 BMC were significantly more effective than control BMC, but after 14 days, there were no differences.

There were no effect of cells on LV remodelling and diastolic function. Both HO-1 BMC and control BMC significantly reduced the infarct size vs. placebo (17.2 ± 2.7 and 18.8 ± 2.5, respectively, vs. 27.5 ± 5.1, p=0.02) in histomorphometry. HO-1-positive donor BMC were detected in the infarct border area in pigs receiving HO-1-cells. No significant differences in expression of inflammatory genes (SDF-1, TNF-α, IL-6, miR21, miR29a and miR133a) in the myocardium were found. In conclusion, intracoronary delivery of allogenic BMC immediately prior to reperfusion improved the LVEF and reduced the infarct size. HO-1 BMC were not superior to control cells after 14 days, however, produced faster recovery of LVEF. Transplanted cells survived in the peri-infarct zone.

Keywords
Reperfusion, myocardial infarction, bone marrow cells, heme oxygenase-1, gene therapy

Background
The default strategy of reperfusion therapy in the acute myocardial infarction (MI) is primary percutaneous coronary intervention (pPCI). Timely pPCI improves the long-term outcome and by restoration of blood flow in the infarct-related artery (IRA) reduces the area of necrosis, improves the left ventricular (LV) function and stops the progression of LV remodelling (1). However, it triggers simultaneously the detrimental cellular and molecular response known as reperfusion injury (RI). RI might be attributed to cause 30–50% of final myocardial necrosis area. Mechanisms of RI are complex, but generally involve the generation of free radicals, intracellular calcium overload and inflammatory response leading to activation of RISK kinase, opening of mitochondrial permeability-transition pore (PTP) and finally to apoptosis and necrosis of cardiomyocytes (2). RI could therefore be one of key factors limiting the benefits of reperfusion therapy in patients with acute MI. So far various pharmacological substances and non-pharmacological approaches (remote preconditioning, ischaemic post-conditioning) have been tested in animal models and small clinical trials yielding equivocal results (2, 3). Recently use of bone marrow (BM)-derived cells has been proposed as adjunct therapy in patients with acute MI. Several clinical trials suggest that BM cells (BMC) might improve the perfusion of peri-infarct area and augment the recovery of LV ejection fraction (4). So far non-selected BM mononuclear cells (MNC), subpopulations of CD34+ cells

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and mesenchymal stem cells (MSC) were used for such experimental therapy without clear evidence of superiority of selected cells over MNC. Acute MI is, however, a clinical scenario in which – in order to target the reperfusion injury – the cells should be available “off the shelf” because pPCI should be done as soon as possible after the symptoms onset. Therefore allogeneic cells seem to be optimal in particular if the population is enriched for cells with angiogenic potential (5). However, the survival of BM cells after intracoronary delivery to the myocardium is reduced due to inflammatory and proteolytic environment in the setting of MI, so it is important to modify the cells in order to increase their resistance against apoptosis. Such property can be achieved by transduction of the BMC with heme oxygenase-1 (HO-1) gene. HO-1 is a key enzyme in metabolism of heme and its products (biliverdin, carbon monoxide, iron) have proven antioxidant, anti-inflammatory, angiogenic and anti-apoptotic properties (6, 7). Its expression is upregulated in the peri-infarct area after MI and HO-1 knockout aggravated ischaemia reperfusion injury in experimental MI (8, 9).

On the other hand, the expression of HO-1 increases the survival of BM cells after myocardial delivery (10). Therefore the combination of cell therapy using allogeneic BMC with gene therapy leading to overexpression of HO-1 might be particularly beneficial in the setting of reperfused MI. The aim of the study was to evaluate the effects of intracoronary delivery of BMC overexpressing HO-1 on the reperfusion injury and LV function in a porcine model of MI.

Methods

Twenty-eight Polish domestic pigs were used (mean weight 38.32 ± 7.56 kg). Experiment has been approved by Ethics Committee of Medical University of Silesia (no 48/2009).

Isolation and culture of BMC

Following protocol was used for the isolation and culture of the BMC. Briefly, BM was harvested from femurs of donor pigs immediately (<2 hours [h]) after sacrifice using the biopsy needle (Medax Medical Services) with 20 ml syringe containing ion-free phosphate-buffered saline (PBS) solution with heparin (0.2U/ml; Polpharma S.A., Poland). Femurs were flushed several times and the cells were transferred to 50 ml Falcon tubes (BD, Franklin Lakes, NJ, USA). Cells suspension was centrifugated (300 x g; 10 minutes [min]; room temperature), and the cellular fraction was resuspended in 10 ml ion-free PBS. BM-MNC were subsequently isolated by Ficoll gradient (Ficoll Paque Plus, GE Healthcare, Waukesha, WI, USA) centrifugation (400 x g; 30 min; room temperature). Buffy coat was transferred to 50 ml Falcon tubes and washed with 10 ml PBS (300 x g; 10 min; room temperature). Cells were resuspended in culture medium (complete EGM2-MV, Lonza, Basel, Switzerland) at the concentration of 10 x 10^6 cells/ml and were seeded on plastic six-well microplates. Culture conditions including plate coating (fibronectin 50 μg/ml + gelatin 2%, 72 h, 37°C) were optimised to achieve BMC fraction enriched with potential endothelial progenitor cells (EPC). Non-adherent fraction of seeded BM cells was discarded with culture medium after 72 h of initial culture and only adherent cell fraction for further propagated. Three ml of EGMN-2MV medium was added to adherent cells and incubated until 80% confluence was achieved. Cells were cultured for three weeks and percentage of potential EPC was assessed based on binding of lectin (Griffonia simplicifolia) and incorporation of Dil-Ac-LDL. Figure 1 shows optimised culture conditions (fibronectin 50 μg/ml + gelatin 2%, 72 h, 37°C) (Fig. 1A, B), which yielded population of BMC enriched for EPC (30–40%). The expression of MSC and EPC markers was confirmed by RT-PCR (CD105, CD29, CD34 and CD31) and in cell culture. RT-PCR was used because of lack of specific antibodies for FACS analysis of these populations in pigs. The expanded BMC underwent three passages and were subsequently divided into three groups: 1) non-transduced cells (control, CTRL-BMC), 2) cells transduced with adenoviral vector (AdV) AdV-GFP (GFP-BMC) and 3) cells transduced with AdV-HO-1 (HO-1 BMC) and used for intracoronary infusion within 48 h after transduction.

Transduction of BMC with HO-1 gene

Adenoviral vector harbouring GFP was kindly provided by Dr. Kazuhiro Oka (Baylor College, Houston, TX, USA) while AdV were a kind gift by Dr. Gisa Tiegs (Erlangen, Germany). Vectors were amplified as described previously (11). Transduction of porcine BMC was performed with adenoviral vector with rat gene encoding for HO-1 (Ad-HO-1) or vector harbouring reporter gene for GFP (Ad-GFP) at 50 MOI. Figure 1 shows cells transduced with AdV with AdGFP. Effectiveness of transduction with AdGFP (control) and AdHO-1 (63.6 ± 4.4%) was measured after 48 h by identification of GFP+ cells using a fluorescence microscope and by RT-PCR confirming the expression of rat HO-1 in porcine cells, respectively. Importantly, staining with Dil-Ac-LDL showed that potential EPC also underwent transduction with adenoviral vector (Fig. 1C, D).

Angiogenic potential of BMC

Angiogenic potential of the BMC was assessed using Matrigel tube formation assay in presence of VEGF (50 ng/ml). Human microvascular endothelial cell (HMEC-1) line and swine kidney epithelial LLC-PK1 cells were used as positive and negative controls, respectively. We showed that expression of HO-1 did significantly increase the angiogenic potential of the HO-1-BMC after 8 h of culture, but the increase was not significant after 20 h, although still a trend to a higher angiogenic potential of HO-1 modified cells was visible (Fig. 2).
Experimental MI with reperfusion

During the adaptation period (4–5 days prior to experiment) all pigs received 200 mg of amiodarone daily (Opacorden, Polpharma SA) in order to reduce the risk of arrhythmia. Animals were premedicated with atropine sulphate (1 mg/20 kg body weight s.c.) and ketamine hydrochloride (Bioketan, 1 ml/10 kg of body weight i.m., Vetfarma- cia, Poland), anesthetised with xylazine hydrochloride (Rometar, 1 ml/10 kg of body weight i.m., Scanvet, Poland) and intubated. General anesthesia was maintained on isoflurane, (Abbott, Abbott Park, IL, USA) and vital functions (electrocardiogram [ECG], blood pressure, O₂ saturation) were measured and recorded every 5 min. Coronary catheterisation was carried out according to previously published protocol (12). Briefly, the right femoral artery was cannulated with 6F vascular sheath and unfractionated heparin (5,000 units) was injected. After intravenous injection of lidocaine (0.1 mg/kg) left coronary artery was cannulated with 6F Judkins right (JR4) guiding catheter and coronary angiography was performed. Diameter of the left anterior descending (LAD) artery was measured by QCA in order to size the occlusion balloon. Coronary guide wire was passed into distal segment of LAD and 10 mm over-the-wire (OTW) balloon catheter with appropriate size to occlude the medial segment of LAD (2.5–3.5 mm) was introduced. Balloon catheter was inflated with pressure sufficient for total occlusion for 60 min. Occlusion was confirmed by contrast injection and ECG changes typical of acute MI (ST-segment elevation). After 60 min the balloon was deflated, which led to reperfusion. In case of severe reperfusion arrhythmia defibrillation was performed immediately.

Study flowchart is shown in Figure 3. Twenty-eight pigs were randomly allocated in 1:1:1:1 manner to either placebo (CTRL, 0.9%NaCl) or actively treated groups which received BMC (1 ml/min) 10 min prior to reperfusion through the inner lumen of the OTW catheter [non-modified BMC (CTRL-BMC), cells transduced with GFP (GFP-BMC) or transduced with rat HO-1 (HO-1 BMC)]. After that catheter was flushed with 5 ml 0.9% NaCl and deflated to achieve reperfusion. Control angiography was done to exclude the thrombosis. After 14 days animals were sacrificed by injection of pentobarbital sodium (Morbital, Biowet, Poland).

**Figure 1:** Preparation of bone marrow cells. A) Scheme of the protocol used to expand BMC enriched in cell fraction with endothelial markers (Lectin, Ac-LDL uptake). B) Optimisation of the protocol revealed that the highest percentage of AcLDL uptaking cells were observed when the first medium change was done 72 h after seeding cells. Double positive Lectin⁺ Dil-AcLDL⁺ cells were detected among BMC expanded with described protocol. C) Cells transduced with AdV were able to uptake AcLDL D) Adenoviral vectors (AdV) encoding for GFP or HO-1 trasngene were able to transduce expanded BM-derived cells, as confirmed by GFP fluorescence and RT-PCR detection of rat HO-1 mRNA.
Transthoracic echocardiography

All animals underwent transthoracic echocardiography (TTE) under sedation performed by experienced veterinarian blinded to the treatment allocation. TTE was done before MI, 30 min and 14 days after the reperfusion using the Aloka 4000 Plus system (Aloka Hitachi, Tokyo, Japan). The following parameters were assessed off-line: left ventricle ejection fraction (LVEF) according to the Simpson’s rule, end-diastolic (EDD) and end systolic diameter (ESD), shortening fraction (SF) and isovolumic relaxation time (RIVRT). In addition, the tissue Doppler imaging (TDI) was performed. Example of M-mode and TDI imaging in control (placebo) and treated animal (HO-1 BMC) is shown in ▶Figure 4.

Laboratory measurements

Venous blood samples were drawn before the procedure, immediately prior to occlusion of LAD, 48 h and 14 days after the reperfusion. Plasma levels of troponin I (TnI), electrolytes (K+, Na+) and complete blood count were performed. ELISA kits for measurement of TnI were species-specific.

Figure 2: Angiogenic potential of bone marrow cells. A) BMC cells formed tubes in in vitro Matrigel assay; however, phenotype of the tubes differed from ones formed by endothelial cells (HMEC). B) Quantification of the number of formed tubes (after 20 h) indicated that HO-1 overexpression enhances tube formation in vitro in comparison to control GFP group after 8 h (p=0.03, non-paired t-test), but not after 20 h.
Histopathology

Infarct size (IA), area-at-risk (AAR) and size of remote non-necrotic myocardium area were measured by histomorphometry according to previously published protocol (1). Slices were measured by planimetry and size of IA, AAR was calculated respective to the LV. Respective samples of the myocardium from IA, AAR and remote myocardium (1.5 mm) were excised and fixed in buffered formaldehyde.

Expression of genes encoding for chemoattractant and inflammatory factors

In samples from IA, AAR and healthy remote myocardium transcriptional activity of following genes was measured by real time RT-PCR stromal-derived factor-1 (SDF-1), tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Elongation factor 2 (EF-2) expression was used as internal control and expression values were calculated as $2^{-\Delta\Delta C_t}$. Additionally, expression of miRNA-21, miRNA-29a and miRNA-133a was checked using the NCode™ VILO™ miRNA cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). U1 snRNA expression was used as internal control.

Presence of donor-derived cells in recipient’s myocardium

Presence of donor cells was assessed by immunofluorescence of GFP (antibody from Abcam, Cambridge, UK; cat.: ab290). Nuclei were stained with DAPI (Vector Labs, Burlingame, CA, USA) and proliferating cells identified with antibody to proliferating cells nuclear antigen (PCNA) (Dako, Glostrup, Denmark; cat.: M0879, clone PC10). In samples from AAR the presence of donor-derived HO-1-positive cells were identified by specific antibody against HO-1 (Santa Cruz Biotechnologies, Santa Cruz, CA, USA; cat.: sc-10789).

Figure 3: Study flowchart.
Statistical analysis

Statistical analysis was carried out using the MedCalc package (MedCalc Software, Mariakerke, Belgium). Normally distributed data were expressed as a mean ± SD, and non-parametric variables as median and range. Differences were compared with ANOVA or Kruskal-Wallis test. P-values of <0.05 were considered statistically significant. Analysis of study power was based on assumption that the difference of at least 10% in mean infarct area between both HO-1-MSC vs. CTRL and HO-1-MSC vs. CTRL-MSC groups was considered significant. IA was calculated on the basis of at least 10 histological sections of the muscle for each animal. Taking into account that the variable was normally distributed (p=0.1 by W-Shapiro-Wilk test), α=0.05 and the number of tissue sections was at least 50 for each group, we estimated that the power is 1 for the former and 0.999 for the latter comparison. Above-mentioned data show that the quantity of animals was sufficient to draw reasonable conclusions.

Results

Study groups were comparable in terms of sex and weight. All animals underwent successful occlusion of LAD. There were nine peri-procedural deaths (32.1%) by VF refractory to defibrillation (five animals from HO-1 BMC, CTRL-BMC and placebo groups and four animals in GFP-BMC group survived). There were no significant differences in frequency of arrhythmia between control and BMC-treated groups.

LV function and remodelling

Baseline values of LVEF and SF were comparable in all groups. Echocardiography carried out 30 min after reperfusion (Fig. 5) showed significantly higher LVEF and SF values in HO-1 BMC in comparison to control cells and placebo group. After 14 days LVEF and SF were higher in all groups receiving BMC than in the placebo group. At this time, there was no superiority of HO-1 BMC in comparison to control cells (Fig. 5A, B). Baseline values of ESD and EDD were comparable in all groups. Thirty minutes after reperfusion there were no significant differences in ESD between treated groups and placebo and EDD values were significantly higher in HO-1 BMC (Fig. 5C-D). Also, after two weeks there were no differences between groups in EDD and ESD values. There were no significant differences in IVRT throughout the experiment (not shown).

Assessment of the infarct size

Figure 6A shows a comparison of IA, AAR and ratio of IA to AAR (IA/AAR). In animals from all groups the AAR was comparable. All groups receiving BMC had a significant reduction of IA versus placebo, but there was no difference between HO-1 BMC and control BMC. Similarly, IA/AAR ratio was lower in groups receiving BMC than placebo without differences between HO-1 and control BMC. There were no differences in baseline levels of TnI as well as measured 48 h (Fig. 6B) and 14 days post MI.
Myocardial expression of inflammatory factors and miRNA

Real time RT-PCR was performed in samples of the myocardium from the IA, infarct border zone and remote myocardium. There were no significant differences in number of mRNA copies for SDF-1, TNF-α and IL-6 in the IA in comparison to the remote myocardium or infarct border zone. The use of HO-1 BMC had no effect on the expression of SDF-1, TNF-α and IL-6 (▶ Fig. 6C). The expression of miR133a, miR-21 and miR-29a showed no regulation in the IA in comparison to the non-infarcted myocardium and there was no effect of BMC on the miRNAs expression (▶ Fig. 6D).

Identification of donor-derived cells in the myocardium

Presence of donor cells was confirmed by identification of GFP+ cells in samples of infarct border-zone. GFP+ cells were identified in the capillary vessels and it co-localised with marker of prolifer-
The study showed that intracoronary infusion of allogeneic BMC enriched with potential EPC ameliorated the RI by a significant reduction of the infarct area measured by histomorphometry 14 days after experimental MI. Observed reduction of IA corresponded with improved LVEF and FS after 14 days in animals receiving BMC. The study showed no clear benefit associated with the use of cells transduced with HO-1 gene with respect to reduction of IA in comparison to control cells. Interestingly in animals treated with HO-1 BMC, the recovery of LVEF and SF was faster than with control cells, because significant improvement was observed already 30 min after reperfusion. However, after 14 days the recovery was similar in all groups. We observed no significant effect of treatment with BM cells on LV remodelling. The mechanisms of this phenomenon are not clear. The RI can be either lethal or present as an evanescent phenomenon.

Discussion

The study showed that intracoronary infusion of allogeneic BMC enriched with potential EPC ameliorated the RI by a significant reduction of the infarct area measured by histomorphometry 14 days after experimental MI. Observed reduction of IA corresponded with improved LVEF and FS after 14 days in animals receiving BMC. The study showed no clear benefit associated with the use of cells transduced with HO-1 gene with respect to reduction of IA in comparison to control cells. Interestingly in animals treated with HO-1 BMC, the recovery of LVEF and SF was faster than with control cells, because significant improvement was observed already 30 min after reperfusion. However, after 14 days the recovery was similar in all groups. We observed no significant effect of treatment with BM cells on LV remodelling. The mechanisms of this phenomenon are not clear. The RI can be either lethal or present as an evanescent phenomenon.

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Figure 6: Histopathology analysis. A) Histomorphometric analysis of the hearts 14 days after MI. Infarct size (IA), area-at-risk (AAR) and size of remote non-necrotic myocardium area were measured by planimetry and calculated respective to the LV. B) Plasma troponin I (TnI) levels measured 48 h after the myocardial infarction. C) RT-PCR analysis of mRNA levels of genes encoding for inflammatory factors (TNF-α, IL-6) and chemotactic factor (SDF-1). D) RT-PCR analysis of miR-21, miR-29a and miR-133a levels.
rhythmia, no-reflow or myocardial stunning and is caused by oxidative stress and inflammation. Early effect of HO-1-positive cells could suggest that HO-1 counteracted above mentioned effects leading to less myocardial stunning because products of HO-1 such as CO, are anti-inflammatory and antioxidative. This explanation is, however, a hypothesis and requires further studies. Also loss of HO-1 activity in the myocardium associated with transient effects of AdV-mediated HO-1 gene expression might explain the modest and temporary effects on LV function.

We observed that HO-1 cells increased LVEDD 30 min after the reperfusion, but had no effect on LV remodelling after 14 days. The mechanism of this observation could involve the early effect on HO-1 on diastolic function; however, there is no direct evidence from this study. So far, no study has investigated effects of BM cells specifically on diastolic function. Sub-studies of human trials such as BOOST showed moderate improvement of diastolic function after BMC therapy (13). A recent paper by Zeiher’s group showed that LV dilatation following MI predicts the diastolic dysfunction in patients treated with BMC (14). Our data showed a trend towards improvement of diastolic function; however, the differences did not reach significance. LVEDD was indeed higher 30 min after the reperfusion and LVESD was lower (although non-significantly). The explanation of this observation could indeed involve the early effect on HO-1 on diastolic function; however, there is no direct evidence from this study.

We have obtained heterogenous population of cells with expression of both EPCs and MSC markers in a 30/70 ratio. Hypothetically such a heterogenous population might be beneficial because of anti-inflammatory and immunomodulating properties of MSC, pro-survival effects of HO-1 and pro-angiogenic effects of EPCs. Safety of such approach has to be verified before clinical translation because available human data confirmed the safety of allogenic MSC, but not EPCs.

Effects of BM-derived MSC transduced with HO-1 gene were assessed in acute MI model in mini pigs by Jiang et al. However, conversely to our model, the cells were administered 1 h after the reperfusion, so the actions of HO-1 could not affect the substantial period of RI. They showed improvement of the LVEF, reduction of ESV and higher capillary density in the peri-infarct area after 14 days, and demonstrated that the effects were reversed by inhibition of HO-1. These effects were more significant in animals treated with MSC expressing HO-1 than with non-modified cells (15). In a similar study Zeng et al. showed that intra-myocardial injection of BM-derived MSC transduced with AdV containing HO-1 1 h after the MI improved LV function, attenuated remodelling and reduced apoptosis to a greater extent than non-modified MSC. Transplantation of HO-1 MSC resulted in the increased expression of HO-1 in the recipients myocardium and increased angiogenesis which was mediated through HO-1 because its inhibition attenuated the increase of capillary formation (16). The effects of HO-1 MSC on LV remodelling and infarct size is probably also mediated by reduction of tissue metaloproteinases (MMP-2, MMP-9) and increased availability of their inhibitors (TIMP-3, TIMP-2) (17).

In the current study, we used allogeneic BMC enriched for po-

Figure 7: Engraftment of bone marrow cells in the myocardium. A) Identification of GFP+ cells in the infarct border-zone 14 days after MI in animals receiving GFP-BMC. Additionally, the expression of PCNA indicates that transplanted cell proliferate. B) Detection of HO-1–positive cells in infarct border zone in animal treated with HO-1 BMC.
tential EPC, which after optimisation of culture conditions constituted about 30% of population. We confirmed increased expression of both GFP and HO-1 after transduction of cells with adenoaviral vectors carrying those genes. Transduction with HO-1 enhanced the angiogenic potential of BMC as evidenced by enhanced tube formation on Matrigel. Accordingly, studies by other groups showed that expression of HO-1 increases the resistance of BMC against oxidative stress and apoptosis as well as paracrine secretion of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF) and transforming growth factor-β (TGF-β), as well as graft survival after intra-myocardial transplantation (10, 18, 19).

The histopathological analysis in our study revealed that, 14 days after the intracoronary infusion, GFP-positive cells could be identified in the recipient’s myocardium. Also the implanted cells expressed PCNA which suggests that they were proliferating. Staining for HO-1 showed the presence of donor cells in the peri-infarct area, which supports the hypothesis that the benefit of the infusion of BMC is mediated by the engraftment of cell in the viable peri-infarct zone. The number of engrafted cells was, however, low and consistently with other studies there is no clear evidence that this form of cell modification might contribute to the substantial increase of cell retention after intracoronary delivery (20, 21). Therefore most likely the effects of intracoronary BMC are dependent on their paracrine function and of salvage of cardiomyocytes in the peri-infarct area which is hypoperfused and stunned but consists of viable cells. Also, animal models as well as clinical studies showed improvement in microvascular function in the peri-infarct area after intracoronary delivery of BMC (22, 23). Li et al. showed that recombinant AAV-mediated HO-1 gene transfer into cardiac myocytes provided enduring protection against RI. In mice model of reperfused MI produced 1 year after the gene transfer, expression of HO-1 significantly reduced IA (24). It was confirmed in HO-1 transgenic mice with significantly attenuated RI and better recovery of LV function after MI (25). Interestingly, in patients with acute MI the plasma levels of HO-1 showed a negative correlation with severity of coronary artery disease further supporting evidence of its protective role in the cardiovascular system (26). Yang et al. used adipose tissue-derived stem cells (ADSC) expressing HO-1 injected intra-myocardially in rabbits 14 days after non-reperfused MI and showed reduced infarct size, improvement of LVEF and less remodelling. In addition, there was substantial differentiation of injected HO-1 ADSC into cardiomyocytes and vascular-like structures after four weeks (27). However, this model reflected chronic infarction, so the mechanisms involved in protection against RI were not assessed. Also pretreatment with HO-1 by AAV-2 vector reduced the infarct area and improved LV function one year after chronic MI in rats. Similar observations were made when the rat model of reperfused MI was used (28, 29).

We measured the expression of chemoattractant SDF-1 and inflammatory cytokines TNF-α, IL-6 in the IA, peri-infarct zone and healthy myocardium and did not find any statistically significant differences. There were also no effects of cell therapy on expression of these markers in the heart. Similarly, we showed no changes of the expression of miR21, miR29a and miR133a after the cell implantation. Conversely, Lakkisto et al. showed that induction of HO-1 in rat heart prior to coronary artery ligation and MI led to increased expression of SDF-1 in the infarcts area (30). Also HO-1 induction as well as the use of CO-donor increased migration of resident c-kit+ cardiac stem cells in the infarct area. CO increased expression of SDF-1, VEGF and VEGFB what suggests that both expression of HO-1 and increased availability of CO are important factors to be considered in therapies targeting HO-1 (30). Jiang et al. showed that HO-1 overexpression in BMC delivered intracoronary increase their homing to the peri-infarct zone and enhance myocardial expression of VEGF, anti-inflammatory IL-10 and reduce the expression of proinflammatory cytokines (31, 32). Induction of HO-1 in cardiac cells by covalt protoporphyrin IX in the rat model of MI led to reduced rate of apoptosis, less fibrosis and more myocyte proliferation in the perinfarct area via downregulation of TGF-β (33). Additionally, up-regulation of HO-1 was involved in the protective effects of simvastatin used in the prevention of RI in cardiac allograft in the rat model as well as cytoprotective effects of hydroxysafflor yellow A in isolated cardiomycocytes (34, 35). Possible mechanisms of HO-1 mediated effects on RI involve the release of CO which activate anti-apoptotic pathways, increase expression of HO-1, Nrf2 and superoxide dismutase preventing opening of PTP (36, 37).

In conclusion, intracoronary delivery of allogeneic BMC enriched for potential EPC immediately prior to reperfusion improved the LVEF and reduced infarct size in pig model. Transduction of BMC with HO-1 was not superior to control cells after 14 days; however, it led to faster recovery of LVEF. Transplanted cells survived in recipients’ myocardium in the peri-infarct zone.

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Conflicts of interest
None declared.

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