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Differential Effects of Progenitor Cell Populations on Left Ventricular Remodeling and Myocardial Neovascularization After Myocardial Infarction

Christophe Dubois, MD,* Xiaoshun Liu, MD, PhD,‖ Piet Claus, PhD,* Glenn Marsboom, PhD,‖ Peter Pokreisz, PhD,‖ Sara Vandenwijngaert, MSc,‖ Hélène Dépelteau, MD,† Witold Streb, MD,* Lertlak Chaothawee, MD,† Frederik Maes, PhD,† Olivier Gheysens, MD, PhD,‡ Zeger Debyser, MD, PhD,¶ Hilde Gillijns, BSc,‖ Marijke Pellens, BSc,‖ Thierry Vandendriessche, PhD,‖ Marinee Chuah, PhD,‖ Desiré Collen, MD, PhD,‖ Erik Verbeken, MD, PhD,§ Ann Belmans, MSc,# Frans Van de Werf, MD, PhD,* Jan Bogaert, MD, PhD,† Stefan Janssens, MD, PhD*‖

Leuven, Belgium

Objectives We compared biological repair after acute myocardial infarction (AMI) with selected porcine progenitor cell populations.

Background Cell types and mechanisms responsible for myocardial repair after AMI remain uncertain.

Methods In a blinded, randomized study, we infused autologous late-outgrowth endothelial progenitor cells (EPC) ($n = 10$, $34 \pm 22 \times 10^6$ CD29-31-positive, capable of tube formation), allogeneic green fluorescent peptide–labeled mesenchymal stem cells (MSC) ($n = 11$, $10 \pm 2 \times 10^6$ CD29-44-90-positive, capable of adipogenic and osteogenic differentiation), or vehicle (CON) ($n = 12$) in the circumflex artery 1 week after AMI. Systolic function (ejection fraction), left ventricular (LV) end-diastolic and end-systolic volumes, and infarct size were assessed with magnetic resonance imaging at 1 week and 7 weeks. Cell engraftment and vascular density were evaluated on postmortem sections.

Results Recovery of LV ejection fraction from 1 to 7 weeks was similar between groups, but LV remodeling markedly differed with a greater increase of LV end-systolic volume in MSC and CON ($+11 \pm 12$ ml/m^2 and $+7 \pm 8$ ml/m^2 vs. -3 ± 11 ml/m^2 in EPC, respectively, $p = 0.04$), and a similar trend was noted for LV end-diastolic volume ($p = 0.09$). After EPC, infarct size decreased more in segments with >50% infarct transmurality ($p = 0.02$ vs. MSC and CON) and was associated with a greater vascular density ($p = 0.01$). Late outgrowth EPCs secrete higher levels of the pro-angiogenic placental growth factor (733 [277 to 1,214] pg/10^6 vs. 59 [34 to 88] pg/10^6 cells in MSC, $p = 0.03$) and incorporate in neovessels in vivo.

Conclusions Infusion of late-outgrowth EPCs after AMI improves myocardial infarction remodeling via enhanced neovascularization but does not mediate cardiomyogenesis. Endothelial progenitor cell transfer might hold promise for heart failure prevention via pro-angiogenic or paracrine matrix-modulating effects. (J Am Coll Cardiol 2010;55:2232–43) © 2010 by the American College of Cardiology Foundation

Cardiac cell transfer is a promising new strategy for biological repair of the dysfunctional heart. Clinical translation of initial proof of concept studies with bone marrow-derived mononuclear progenitor cells in mice (1) has gained significant momentum, but studies in patients with acute myocardial infarction (AMI) have shown mixed results (2–4). In

From the *Division of Cardiology, Department of Cardiovascular Diseases, and the Departments of †Radiology, ‡Nuclear Medicine, and §Medical Diagnostic Sciences, Gasthuisberg University Hospitals Leuven, Leuven, Belgium; ‖Vesalius Research Center, Flanders Institute for Biotechnology (VIB); and the Departments of ¶Molecular Medicine and #Public Health, Biostatistical Center, KU-Leuven, Leuven, Belgium. This work was supported by grants of the Fund for Scientific Research–Flanders G.0442.06 and G.0280.05, VIB (Dr. Janssens) and the Research Fund KU-Leuven GOA/2007/13 (Drs. Janssens and Pokreisz). Dr. Janssens is holder of a chair supported by AstraZeneca. Dr. Dépelteau was supported by a fellowship, funded by the Royal College of Physicians and Surgeons of Canada.

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most randomized studies a heterogeneous mononuclear cell (MNC) population was infused in the infarct-related coronary artery (IRA), and change in global ejection fraction was evaluated as a surrogate primary efficacy end point.

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There is a growing recognition that indirect paracrine trophic effects rather than cardiomyogenesis resulting from progenitor cell transdifferentiation mediate the observed changes in contractile function or infarct size after infusion of a mixed MNC population (5). Although critically important variables, including doses and delivery routes of labeled mixed mononuclear or mesenchymal (MSC) progenitor cells, have been investigated in representative large animal models (6–10), direct comparison of selected cell populations has received limited attention. Therefore we compared functional and structural recovery after AMI in pigs after intracoronary (IC) infusion of autologous late outgrowth endothelial progenitor cells (EPCs) versus allogeneic MSCs.

Methods

Study design. The Animal Care and Use Committee of University of Leuven approved this study. At 1 week after AMI, pigs were randomly assigned to blinded IC delivery of control medium (CON), autologous EPCs, or allogeneic MSCs. Hemodynamic variables were measured at baseline and after 1 week and 7 weeks. Magnetic resonance imaging (MRI) was performed at 1 week and 7 weeks after AMI in a subgroup of pigs. All pigs were killed at 7 weeks. Growth-related changes in myocardial mass and volumes were evaluated in a separate series of healthy pigs over the same time period.

Cell preparation. ISOLATION AND CULTURE OF AUTOLOGOUS LATE-OUTGROWTH EPCs. Mononuclear cells were isolated from 40 ml blood by Lymphoprep density centrifugation (Axis-Shield, Oslo, Norway) and 50 to 100 $\times$ 10^6 MNCs were plated on fibronectin-coated (5 μg/ml) dishes in EBM2 (Clonetics, San Diego, California), supplemented with 5% FBS (Invitrogen, Carlsbad, California), vascular endothelial growth factor, human epidermal growth factor, human basic fibroblast growth factor, ascorbic acid, R3-insulin-like growth factor-1, and gentamycin/amphotericin-B. After 4 days, nonadherent cells were removed and cells were cultured for an additional 3 weeks. Late-outgrowth EPCs were then incubated for 24 h with iron oxide (Endorem, Guerbet, France) and poly-L-lysine (Sigma) and diluted in phosphate-buffered saline for phenotypic characterization and IC delivery. Alternatively, for biodistribution studies, EPCs were genetically labeled with a lentiviral reporter construct (p24 titer 5.5 $\times$ 10^6 pg/ml, 1/150 dilution) expressing nuclear targeted beta-galactosidase (LacZ). Expression of surface markers was performed by immunofluorescence and flow cytometry (CD31, CD45, CD29, and SLA DR).

ISOLATION AND CULTURE OF ALLOGENEIC MSCs. Bone marrow was aspirated from the femur (10 ml), diluted in phosphate-buffered saline, 1:3, and filtered. The MNC fraction was isolated with Lymphoprep (Axis-Shield) and amplified in adherent cell cultures (0.1% gelatine-coating). Phenotypic characterization of MSCs was performed by evaluating expression of surface markers (CD31, CD90, CD29, CD44, and CD45), and plasticity was confirmed with adipogenic and osteogenic differentiation (stem cell technology) (11,12). The MSCs were genetically labeled with lentiviral vectors encoding enhanced green fluorescent peptide (GFP) or nuclear targeted LacZ gene for biodistribution studies. The functional titer of the GFP-expressing lentiviral vector was 1.75 $\times$ 10^7 U/ml; multiplicity of infection was 14.

Induction of AMI and hemodynamic measurements. Pigs (20 to 30 kg) were pre-treated with 400 mg amiodarone for 1 week and with aspirin and clopidogrel 1 day before AMI. Pigs were pre-anesthetized with ketamine hydrochloride (20 mg/kg, IM) (Anesketin, Eurovet, Heusden, Belgium), followed by continuous infusion of propofol (0.15 mg/kg/min, IV), intubated and ventilated with a 1:1 mixture of air and oxygen and anticoagulated (10,000 IU heparin). The AMI was induced under continuous electrocardiographic (ECG) monitoring by stenting and 90-min balloon occlusion of the proximal circumflex artery, followed by reperfusion. One week later, control medium, EPC, or MSC were injected via a perfusion catheter inflated in the stented circumflex artery segment during a 10-min stop-flow condition. Immediately after cell transfer and before pigs were killed, angiography of the IRA was repeated to confirm patency. Aspirin (100 mg) and clopidogrel (75 mg) were given daily for 7 weeks.

Hemodynamic variables were measured with a 7-F Millar catheter positioned in the left ventricle (LV). Heart rate, LV systolic and diastolic pressures, and first derivatives thereof ($dP/dt_{max/min}$) were recorded at baseline, before cell transfer,

Abbreviations and Acronyms

AMI = acute myocardial infarction
CON = vehicle, control medium
ECG = electrocardiographic
EDV = end-diastolic volume
EPC = endothelial progenitor cell
ESV = end-systolic volume
GFP = green fluorescent peptide
IC = intracoronary
IGF = insulin-like growth factor
IRA = infarct related artery
LacZ = beta-galactosidase
LV = left ventricle/ventricular
LVEDV = left ventricular end-diastolic volume
LVESV = left ventricular end-systolic volume
MMP = matrix metalloproteinase
MNC = mononuclear cell
MRI = magnetic resonance imaging
MSC = mesenchymal stem cell
PLGF = placental growth factor
qPCR = quantitative polymerase chain reaction
TGF = transforming growth factor
TTC = 2,3,5-triphenyltetrazoliumchloride
VEGF = vascular endothelial growth factor