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Long-acting beneficial effect of percutaneously intramyocardially delivered secretome of apoptotic peripheral blood cells on porcine chronic ischemic left ventricular dysfunction☆

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ABSTRACT

The quantity of cells with paracrine effects for use in myocardial regeneration therapy is limited. This study investigated the effects of catheter-based endomyocardial delivery of secretome of 2.5×10^9 apoptotic peripheral blood mononuclear cells (APOSEC) on porcine chronic post-myocardial infarction (MI) left ventricular (LV) dysfunction and on gene expression. Closed-chest reperfused MI was induced in pigs by 90-min occlusion followed by reperfusion of the mid-LAD (day 0). At day 30, animals were randomized to receive porcine APOSEC ($n = 8$) or medium solution (control; $n = 8$) injected intramyocardially into the MI border zone using 3D NOGA guidance. At day 60, cardiac MRI with late enhancement and diagnostic NOGA (myocardial viability) were performed. Gene expression profiling of the infarct core, border zone, and normal myocardium was performed using microarray analysis and confirmed by quantitative real-time PCR. Injection of APOSEC significantly decreased infarct size ($p < 0.05$) and improved cardiac index and myocardial viability compared to controls. A trend towards higher LV ejection fraction was observed in APOSEC vs. controls ($45.4 \pm 5.9\%$ vs. $37.4 \pm 8.9\%$, $p = 0.052$). Transcriptome analysis revealed significant downregulation of caspase-1, tumor necrosis factor and other inflammatory genes in APOSEC-affected areas. rtPCR showed higher expression of myogenic factor Mefc2 ($p < 0.05$) and downregulated caspase genes ($p < 0.05$) in APOSEC-treated pigs. In conclusion, overexpression of MEF2c and repression of caspase was related to decreased infarct size and improved cardiac function in secretome-treated animals. Altered gene expression 1-month post-APOSEC treatment proved the long-acting effects of cell-free therapy with paracrine factors.

1. Introduction

Although great effort has been made to replace infarcted myocardium with regenerative cells, the current methods have shown marginal efficacy in clinical applications [1–5]. According to the 'dying stem cell' hypothesis, regenerative stem cells are already undergoing apoptosis even as they are being delivered into the ischemic myocardium [4]. Thus, the best way to attenuate infarction may be to induce immunomodulatory mechanisms in a

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paracrine manner [5–8]. We have shown previously that the secretome of apoptotic peripheral blood cells (APOSEC), which is derived from large numbers of easily-obtainable peripheral blood mononuclear cells (PBMCs), can be used to therapeutically regenerate the myocardium after acute ischemic injury [9–12].

The optimal mode of delivering reparative cells or substances into the ischemic myocardium (i.e. intracoronary or percutaneous intramyocardial delivery) is still a matter of debate. Intracoronary delivery of cells increases the risk of temporary occlusion of the infarct-related artery and requires active migration of the cells from the vessel lumen to the injured tissue site [13]. The inherent disadvantages of cell therapy include the obvious limitations of the relatively small number of autologous adult cells that are available and the relatively large injection volume of the cells, especially for intramyocardial application [14,15]. Using APOSEC, which contains a mixture of cytokines and growth factors, might avoid the disadvantages of cell-based therapies and be more feasible while also enhancing therapeutic efficiency.

Due to the early death of many regenerative cells when injected into the necrotic core, intramyocardial regenerative therapies instead target the infarct border zone, which shows signs of hibernating myocardium. Interestingly, some reports have described the metabolic, proteomic, and genomic differences between the infarct core and remote (normal) myocardium, but there is no genomic information about this special border zone of infarction [16,17]. Furthermore, no studies have investigated the effects of secretome-based therapies on gene expression in the core and border zone of myocardial infarction (MI).

We hypothesized that percutaneous intramyocardial delivery of APOSEC would be safe and effective in a clinically relevant porcine model of chronic left ventricular (LV) dysfunction after MI. We further hypothesized that the trophic effect of APOSEC could cause structural changes, leading to improved hemodynamic function post-MI via altered gene expression. In our experiments we have injected APOSEC or placebo locally intramyocardially into the border zone of infarction using the catheter-based 3D NOGA-guided intramyocardial application method and investigated the LV function, infarct size and gene expression of the ischemic injured myocardium.

2. Materials and methods

2.1. Porcine model of acute MI and APOSEC production

This study used a porcine closed-chest reperfused acute MI model. Domestic pigs ($n = 22$) underwent percutaneous 90-min balloon occlusion of the mid-left anterior descending coronary artery (LAD) followed by reperfusion (Fig. 1 and Supplementary Materials and Methods). APOSEC was produced from pig PBMCs (Supplementary Materials and Methods). Animal investigations were carried out in accordance with the "Position of the American Heart Association on Research Animal Use," as adopted by the AHA on November 11, 1984. The study was approved by the Ethics Committee on Animal Experimentation at the University of Kaposvar, Hungary.

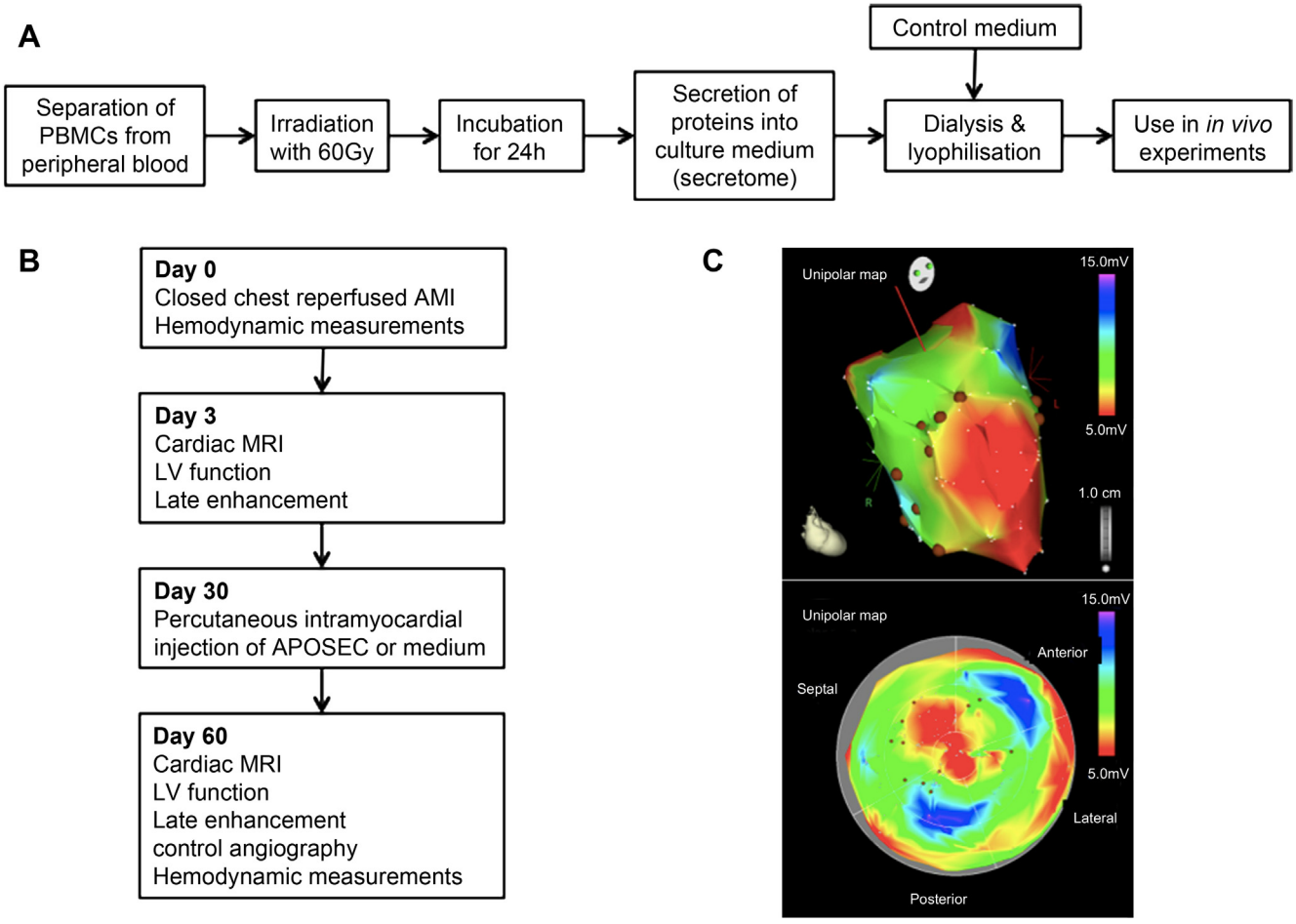

Fig. 1. Study design. A. Schematic showing how APOSEC is prepared from peripheral blood mononuclear cells (PBMCs). B. Study design. C. 3D views of the left ventricle derived by NOGA endocardial mapping and NOGA-guided injection into the border zone of chronic infarction. Brown dots mark the locations of the intramyocardial injections in the border zone of infarction. Color-coded 3D image (upper) were converted to a 2D polar map (below). Red represents non-viable infarcted myocardium, while green and yellow indicated the border zone of infarction. Normal viable myocardium is colored blue and purple.