Methylglyoxal-Derived Advanced Glycation End-products in Myocardial Infarction

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Introduction:

Myocardial infarction (MI) stimulates the rapid production of advanced glycation end products (AGEs). Collagen, and particularly its arginine sites, is a major target for glycation by methylglyoxal (MG), which is a key glycating agent. These sites are required for proper cell adhesion and survival. Therefore, a modified extracellular matrix (ECM) post-MI may negatively affect: 1) the heart's endogenous repair responses; and 2) the efficacy of cell-based regenerative therapies. For example, this may affect circulating angiogenic cells (CACs) that are recruited to the myocardium in response to MI, and which are also the principal cell population evaluated so far in clinical cell therapy trials. In this work, we sought to: 1) examine the *in vivo* effect of MG accumulation post-MI using a transgenic mouse model that over-expresses glyoxalase-1 (GLO1; a MG metabolizing enzyme); and 2) understand the effects of AGE-modified collagen on the function of CACs *in vitro*. We hypothesized that MG plays an important role in the remodeling and functional changes that occur post-MI; and that MG-modified type I collagen impairs CAC function, and thus may contribute to the minimal endogenous repair responses seen post-MI. This provides a rationale for the application of biomaterials to restore critical ECM signaling needed for effective regeneration of the infarcted heart.

Materials and Methods:

All animal procedures were approved by the University of Ottawa Animal care committee, in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. Briefly, MI was induced in *hGlo1*^{overexp} transgenic mice (C57BL/6J background) and their wild-type (WT) littermates. Heart function was assessed via echocardiography at weeks 1 and 4 post-MI, with mice being subsequently sacrificed for tissue histology analysis. For *in vitro* studies, total peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors after obtaining informed consent (approved by the Human Research Ethics Board of the University of Ottawa Heart Institute). PBMCs were cultured on fibronectin for 4 days to generate the CAC population. CACs were subsequently cultured on type I collagen I that had been treated overnight with PBS, 1mM or 10mM methylglyoxal. Cells were then lifted for cell adhesion, chemotaxis, apoptosis, and angiogenesis assays. Cell phenotype was also analyzed using a FACSAria™ flow cytometer. All statistical analysis was conducted using IBM SPSS. Comparisons between two groups were performed using a student's two-tailed t-test. Comparisons between multiple groups were performed by one-way ANOVA with a post-hoc Tukey test.

Results:

Baseline GLO1 activity (as measured by S-D-lactoylglutathione formation) was greater in *hGlo1*^{overexp} mice (2.1±0.1 mmol/minute/mg cellular protein) compared to the WT mice (1.4±0.1;

$p<0.0001$). Left ventricular ejection fraction (LVEF) at 1 week post-MI was not different between groups. However, at 4 weeks post-MI, LVEF in the *hGlo1*^{overexp} mice (49.7±2.9%) was significantly greater than in WT mice (31.1±0.6%; $p<0.003$). Furthermore, vascular density was higher in sections of heart tissue from *hGlo1*^{overexp} mice (11.0±1.4, for arterioles/FOV) compared to WT mice (4.5±0.8, $p<0.001$). *In vitro,* SDS-PAGE and Western Blot analysis revealed the modification of collagen by MG treatment, which was confirmed by using the 1G7H5 antibody, which is specific for the hydroimidazolone product (MG-H1) of MG glycation. Co-incubation of collagen with MG and a MG-specific scavenger, aminoguanidine (AG), prevented MG-H1 formation. We show that collagen glycation may play a significant role in the lack of therapeutic effect of mobilized pro-angiogenic CACs. Specifically, we observed: 1) reduced adhesion (53% reduction compared to control, $p<0.05$); 2) increased susceptibility to hypoxia-induced apoptosis (43% increase compared to control, $p<0.05$); 3) altered phenotype through reduction of pro-angiogenic progenitors $CD34^+$ and $CD133^+$ (75% and 44% reduction, respectively, compared to control, $p<0.005$); and 4) decreased capacity to mediate *de novo* vasculogenesis (1310±202 and 2150±212, for total network length (μm) $p<0.005$) of CACs cultured on MG-modified vs. normal collagen.

Discussion:

We demonstrate that MG may contribute significantly to the pathogenesis of MI. Although MG can have multiple targets, MG-mediated damage to the ECM, notably type I collagen, may play a role in the impaired endogenous repair response. This axis may also have important implications on the efficacy of regenerative therapy for cardiac regeneration. Therefore, intervening and potentially restoring ECM signaling in the infarcted tissue may have the dual benefit of improving the heart's natural ability to repair while making the hostile cardiac environment more amenable to other therapies. A better understanding of modifications occurring post-MI to type I collagen and other ECM proteins, and how biomaterials can intervene, may prove important to meeting the clinical challenges of regenerating the infarcted heart.