Effect of Physiologic Oxygen Tension Cultured Cardiac Mesenchymal Cells on Structural Improvement in Failing Hearts

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ABSTRACT

Stem/progenitor cells are usually cultured at atmospheric O_2 tension (21%). Since physiologic O_2 tension in the heart is ~5%, using 21% O_2 may cause oxidative stress and toxicity. Cardiac mesenchymal cells (CMCs), a newly discovered and promising type of progenitor cells, are effective in improving left ventricular (LV) function after myocardial infarction (MI). We have previously shown that, compared with 21% O_2, culture at 5% O_2 increases CMC proliferation, telomerase activity, telomere length, and resistance to severe hypoxia *in vitro* and results in greater functional improvement in the failing heart (two independent assayed by echocardiography and hemodynamics) at 35 days after cell transplantation *in vivo*. However, the effects of 5% O_2 cultured CMCs on the structure of the failing heart are unknown. Therefore, murine CMCs were cultured at 21% or 5% O_2. Mice with heart failure caused by a 60-min coronary occlusion followed by 30 days of reperfusion received vehicle, 21% or 5% O_2 CMCs via echocardiography-guided intraventricular injection (Figs. 1 - 3). After 35 days, compared with vehicle group, the 5% O_2 CMC-treated hearts exhibited a decrease in scar size and a concomitant increase in the amount of viable myocardium in the risk region (assessed by trichrome staining) (Fig. 4), concomitant with an increase in LV anterior wall (infarct wall) thickness, a decrease in LV posterior wall (noninfarcted wall) thickness, and a decrease in LV expansion index (indicative of reduced LV dilatation). In contrast, the 21% O_2 CMC-treated hearts did not differ significantly from the vehicle-treated hearts with respect to scar size, viable myocardium, or noninfarcted wall thickness. As shown in Figure 5 (picrosirius red staining and polarized light microscopy), at 35 days after cell therapy, collagen content in the heart was not significantly different in the 21% O_2 CMC group compared with the vehicle group. In contrast, collagen content was significantly reduced in hearts treated with 5% O_2 grown CMCs as compared with vehicle group (40.1±3.8% of risk region vs. 51.6±4.7%, $P<0.05$; 9.4±2.1% of noninfarcted region vs.15.7±1.9%, $P<0.05$ – a relative reduction of nearly 50%). 5% O_2 CMCs, but not 21% O_2 CMCs, significantly decreased scar size, increased viable myocardium, reduced LV dilatation, and limited myocardial fibrosis both in the risk and noninfarcted regions of failing hearts. These data indicate, for the first time, that culturing CMCs at physiologic (5%) O_2 tension provides superior therapeutic efficacy in ameliorating cardiac remodeling via morphologic improvement in the failing heart.

RESULTS

Experimental Protocol

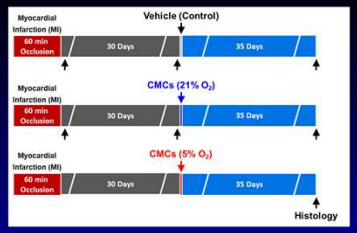
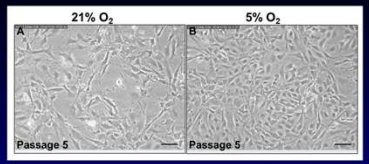


Fig. 1

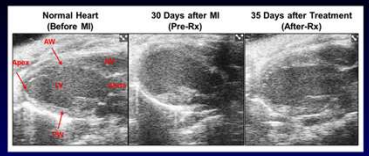
Cardiac Mesenchymal Cells (CMCs) Cultured at 21% vs. 5% Oxygen Tension



Representative microscopic images of CMCs acquired from 21% (**A**) or 5% (**B**) cultured oxygen tension at passage five under magnification x 200. Scale bars, 100 µm.

Fig. 2

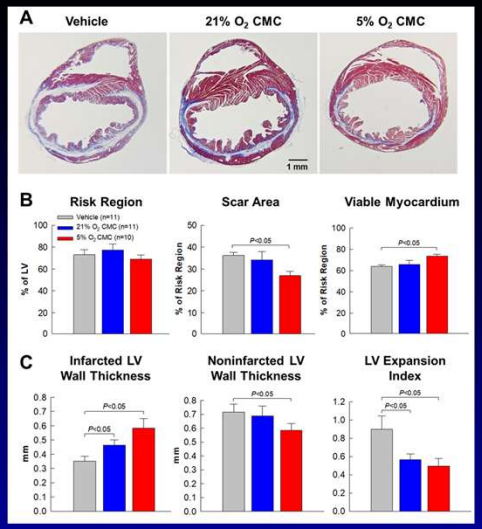
Effect of CMCs Cultured at 5% O_2 Tension on Cardiac Function of Failing Hearts



Representative echocardiography B-mode long-axis images obtained before MI (left), 30 days after MI (Pre-Rx) (middle) and 35 days after 5% O_2 cultured CMC treatment (After-Rx) (right). LV, left ventricle; RV, right ventricle; AW, anterior wall; PW, posterior wall.

Fig. 3

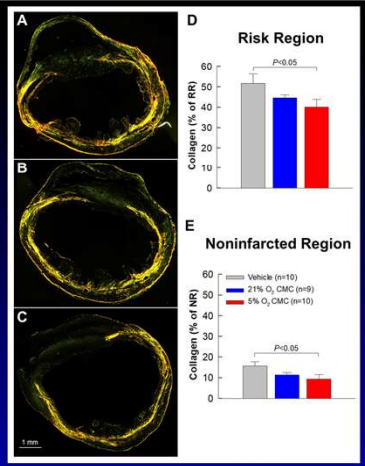
Effect of CMCs Cultured at 5% O_2 Tension on Structural Improvement in Failing Hearts



A. Representative Masson's trichrome-stained myocardial sections from vehicle, 21% O_2 CMC, or 5% O_2 CMC-treated hearts. Scar tissue and viable myocardium are identified in blue and red, respectively.

B and C. Quantitative analysis of left ventricle morphometric parameters. Data are mean ± SEM.

Fig. 4



Effect of CMCs Cultured at 5% O_2 Tension on Collagen Content in Failing Hearts after 35 Days of Cell Therapy

A–C. Representative images of left ventricle sections stained with picrosirius red and analyzed with images acquired under polarized light microscopy from vehicle, 21% O_2 CMC, or 5% O_2 CMC-treated hearts.

D and E. Quantitative analysis of collagen content in myocardium expressed as a percentage of the risk region (scar area plus scar border zones) (**D**) or non-infarcted region (**E**). Data are mean ± SEM.

Fig. 5