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Biological properties of cardiac mesenchymal stem cells in rats with diabetic cardiomyopathy

Diógenes Rodrigo Maronezzi de Paula[a], Vanessa Capuano[a], Daniel Mendes Filho[a], Anna Cecília Dias Maciel Carneiro[b], Virgínia de Oliveira Crema[b], Lucas Felipe de Oliveira[a,d], Aldo Rogélis Aquiles Rodrigues[a,d], Nicola Montano[c], Valdo José Dias da Silva[a,d,*]

[a] Department of Physiology, Biological and Natural Sciences Institute, Triangulo Mineiro Federal University, Uberaba, MG, Brazil
[b] Department of Structural Biology, Biological and Natural Sciences Institute, Triangulo Mineiro Federal University, Uberaba, MG, Brazil
[c] Department of Clinical Sciences and Health Community, Ospedale Maggiore Policlinico, University of Milan, Milan, Italy
[d] National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, RJ, Brazil

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ABSTRACT

Cardiomyopathy is a major outcome in patients with diabetes mellitus (DM) and contributes to the high morbidity/mortality observed in this disease.

Aims: To evaluate several biological properties of cardiac mesenchymal stem cells (cMSCs) in a rat model of streptozotocin-induced DM with concomitant diabetic cardiomyopathy.

Main methods: After 10 weeks of DM induction, diabetic and control rats were assessed using ECG and ventricular hemodynamics monitoring. Then, the hearts were excised and processed for histology and for extracting non-cardiomyocytic cells. A pool of these cells was plated for a colony forming units-fibroblasts (CFU-F) assay in order to estimate the number of cMSCs. The remaining cells were expanded to assess their proliferation rate as well as their osteogenic and adipogenic differentiation ability.

Key findings: DM rats presented intense hyperglycemia and changes in ECG, LV hemodynamic, cardiac mass index and fibrosis, indicating presence of DCM. The CFU-F assay revealed a higher number of cardiac CFU-Fs in DM rats (10.4 ± 1.1 CFU-F/10^5 total cells versus 7.6 ± 0.7 CFU-F/10^5 total cells in control rats, $p < 0.05$), which was associated with a significantly higher proliferative rate of cMSCs in DM rats. In contrast, cMSCs from DM rats presented a lower capacity to differentiate into both osteogenic ($20.8 \pm 4.2\%$ versus $10.1 \pm 1.0\%$ in control rats, $p < 0.05$) and adipogenic lineages ($4.6 \pm 1.0\%$ versus $1.3 \pm 0.5\%$ in control rats, $p < 0.05$).

Significance: The findings suggest, for the first time, that in chronic DM rats with overt DCM, cMSCs increase in number and exhibit changes in several functional properties, which could be implicated in the pathogenesis of diabetic cardiomyopathy.

1. Introduction

Diabetes mellitus (DM) is metabolic disorder with several etiologies; it is characterized by chronic hyperglycemia that results from a deficiency in insulin secretion and/or function [1–3]. The cardiovascular system is affected by diabetes and hyperglycemia, leading to the development of severe cardiovascular disorders with high morbidity/mortality [4,5]. Large arteries and microcirculation are particularly affected by chronic hyperglycemia, causing or accelerating conditions such as coronary and cerebral artery disease, retinopathy, nephropathy and neuropathy. In addition to circulatory vessels, DM can directly affect specific tissues, such as the myocardium [6].

In 1972, Rubler et al. described four patients with DM and congestive heart failure without any signs of arterial hypertension or coronary artery disease. Upon necropsy, the anatomic dissection of their hearts revealed left ventricular hypertrophy and fibrosis without any evidence of coronary atheroma or other cardiac pathology. This new clinical entity was considered independent and was named diabetic cardiomyopathy (DCM) [7]. Clinically, DCM is characterized by the presence of diastolic and/or systolic left ventricular dysfunction in DM patients in the absence of systemic arterial hypertension, coronary arterial disease and any other structural cardiac diseases [8–11]. Functionally and pathologically, DCM is characterized by abnormal myocardial metabolism, ventricular contractile dysfunction, higher

* Corresponding author at: Department of Physiology, Biological and Natural Sciences Institute, Triangulo Mineiro Federal University, Praca Manoel Terra, 330, Uberaba, MG 38025-015, Brazil.
E-mail address: valdo@mednet.com.br (V.J.D. da Silva).

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ventricular wall stress that leads to heart failure and cardiomyocyte hypertrophy, reparative and reactive fibrosis, and death [12–14].

Cardiac mesenchymal stem cells (cMSCs) were first described by Hoogduijn et al.; the group isolated human cMSCs and demonstrated their ability to differentiate into osteogenic and adipogenic lineages as well as their immunomodulatory properties [15]. Chong et al. observed these cells in fetal and adult mouse hearts and suggested their probable proepicardial organ origin and perivascular localization [16]. These cMSCs do not express c-Kit and are positive for pericyte ($CD146^+$) and fibroblast (vimentin and other surface fibroblast proteins) markers and possess the characteristics of bone marrow-derived MSCs, including comparable morphology and similar antigen expression (CD105, CD73, CD29 and CD44) [17]. cMSCs seem to also present some characteristics associated with their cardiac tissue origin, making them an important and unique cell source for efficient cardiac repair [18].

Even though several studies have shown the damaging effects of hyperglycemia in cardiomyocytes and other cardiac stem cells, there have been no reports in the literature regarding the cMSC functional changes induced by hyperglycemia or diabetic cardiomyopathy. Therefore, the present study aimed to evaluate the effects of DM (hyperglycemia) and diabetic cardiomyopathy in a streptozotocin-induced DM Wistar rat model on the number of cMSCs as well as on several functional properties of cMSCs. The results implicate cMSCs in the pathogenesis of diabetic cardiomyopathy.

2. Methodology

2.1. Experimental animals and groups

Forty-four male 20-week-old Wistar rats weighing 300–350 g were kept under controlled and stable conditions (temperature 22 °C, humidity 40–70% and light-dark cycle of 12/12 h) with free access to water and feed. All animals were obtained from the animal facilities of the Department of Physiology at the Federal University of Triangulo Mineiro (UFTM) in Uberaba/MG, Brazil and received humane care in accordance with the ethical principles of animal experimentation adopted by the Brazilian College of Animal Experimentation (CONCEA), and the protocol was approved by the UFTM Ethics Committee on Animal Use (Protocol number: 404).

The studied animals were allocated into two experimental groups: (i) the control non-diabetic group (total number = 20) and (ii) the diabetes mellitus (DM) group (total number = 24). DM was induced via intravenous injection (penian vein) of streptozotocin (50 mg/kg, Sigma-Aldrich Corp.) in overnight fasted and previously anesthetized (tribromoethanol, Sigma-Aldrich Corp., 250 mg/kg, via intra-peritoneal injection) animals. DM developed for 10 weeks during which both blood glucose (measured from caudal peripheral blood via glucosticks and a glucometer — Roche Diagnostics) and body weight were evaluated weekly. At 10th week, in order to confirm the presence of DCM in the DM animals, functional (electrocardiogram and left ventricular hemodynamics) and structural evaluation (histological study) were performed. To certify the presence of DCM after 10 weeks of DM induction, an individual DM rat might be positive for at least four out five following parameters: abnormal ECG or LV hemodynamics, fibrosis, presence of focal necrosis areas and inflammatory infiltration.

2.2. Electrocardiographic recordings

Prior to the DM induction and on the last day after the 10-week follow up, the animals from the control and DM groups underwent baseline electrocardiogram (ECG) recordings. The rats were anesthetized with tribromoethanol (250 mg/kg, via intra-peritoneal injection) and placed in the supine position on a table for ECG recording using a portable six-channel electrocardiograph (Model ER-661, Medicor, Budapest, Hungary) coupled to a computerized data acquisition system (DI-720-USB, DATAQ Instruments, Akron, OH, USA). The sampling rate of the signal acquisition was 1000 Hz. Small needle electrodes were connected to the animals at established standard positions for the leads of the frontal (D1, D2, D3, aVR, aVL, and aVF) and horizontal plane (V1, V2, V3, V4, V5, and V6). The following parameters were evaluated: RR interval (RRi), P-wave duration (Pd), PR interval (PRi), QRS-wave duration (QRSd), QT interval (QTi), and corrected QT interval (corQTi), which was the QT interval corrected for RR interval (RR) by Bazett's formula: $corQTi = QTi\ (s) / [RRi\ (s)]^{1/2}$. All tracings were analyzed by a single blinded evaluator. The 95th percentiles (P95) of all ECG parameters from control non-diabetic group were calculated. These P95 values were taken as cut-off values in order to identify abnormal values of ECG parameters in DM rats.

2.3. Left ventricular hemodynamics

At the end of the last ECG recording session, 17 animals (8 controls and 9 DN rats) were reanesthetized (sodium thiopental, 40 mg/kg, via intra-peritoneal injection), placed in the dorsal supine position on a surgical table, and submitted to a surgery consisting of a median cervicotomy and dissection of the right carotid artery to implant a cannula (polyethylene tube, PE-50, Clay Adams Brand) into the left ventricle through the right common carotid artery. The cannula was then connected to a pressure transducer and coupled to a computerized data acquisition system (DI-720-USB, DATAQ Instruments, Akron, OH, USA) for continuous left ventricular pressure recording for 5–10 min. From the left ventricular (LV) pressure signal, the peak systolic pressure and heart rate, as well as the first positive and the first negative derivative of LV pressure were calculated, and the maximum LV dP/dt (dP/dtmax, maximal velocity of contraction) and the minimum LV dP/dt (dP/dtmin, maximal velocity of relaxation) were measured in order to verify the presence of systolic and diastolic mechanical dysfunction, respectively. The 5th percentiles (P5) of all LV hemodynamics parameters from control non-diabetic group were calculated. These P5 values were taken as cut-off values in order to identify abnormal values of LV hemodynamics parameters in DM rats.

2.4. Evaluation of cardiac hypertrophy by cardiac mass index

Animals were weighed after euthanasia with an overdose of anesthesia. Then, the hearts were excised and weighed to calculate the cardiac mass index, which is the ratio of the absolute heart weight (in mg) to the animal body weight (in g).

2.5. Extraction of non-cardiomyocytic cells from the hearts

After euthanasia and evaluation of cardiac mass index, hearts were longitudinally sectioned in two halves (four-chamber sections). One half was used for extraction of cardiac cells, and another one for histology. For the extraction of cardiac cells, enzymatic digestion of fresh heart tissue was performed. The hearts were sectioned into very small pieces and treated with trypsin-EDTA 0.25% in a water bath (37 °C for 10 min). After this process, the solution was centrifuged twice. After the last centrifugation, the obtained pellets were resuspended and then filtered through a 70 μm cell strainer (BD Biosciences, USA). The cells were counted in a Neubauer chamber using light microscopy. After they were counted, the cells were cultured in six-well plates in low-glucose DMEM containing 10% fetal bovine serum, 200 mM L-glutamine and 1%, penicillin/streptomycin in order to perform the colony forming unit-fibroblasts (CFU-F) assay, which allows for an indirect estimation of the relative number of mesenchymal stem cells present in the cell sample, or were plated at a high cell density (2×10^6 cells/well) until the 5th passage in order to estimate the in vitro proliferation rate or evaluate the osteogenic or adipogenic differentiation ability. Cardiac mesenchymal stem cells at the 5th passage were chosen because at this passage the number of contaminant monocytes/macrophage cells is minimum and the spindle-shaped morphology, representing cells with

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