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Original Article

Galectin-3 is expressed in the myocardium very early post–myocardial infarction

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ABSTRACT

Background: Galectin-3 (GAL-3) plays a regulatory role in several diverse biological processes and disease states. It is associated with heart failure and increased risk of death in a number of studies. We aim to study the direct effects of ischemia on GAL-3 levels in the heart very early in the course of events following myocardial infarction (MI).
Methods: Male C57B6/J mice were used for permanently ligating the left anterior descending artery of the heart to create ischemia/infarction in the anterior wall of left ventricle (LV). Heart samples were processed for immunohistochemical and immunofluorescent labeling, enzyme-linked immunosorbent assay, and quantitative reverse transcriptase polymerase chain reaction to identify GAL-3 levels in the heart during the first 24 h following MI.
Results: GAL-3 mRNA was significantly increased at 60 min ($P=.032$), 4 h ($P=.012$), and 24 h ($P=.00$) post-MI groups in the infarcted LV as compared to sham. Thirty minutes post-MI GAL-3 mRNA is higher than the sham and almost reaching statistical significance ($P=.056$). GAL-3 protein was significantly increased in the LV at 30 min ($P=.021$), 60 min ($P=.029$), 4 h ($P=.015$), and 24 h ($P=.01$) post-MI compared to corresponding sham-operated mice. Plasma GAL-3 levels are also significantly raised at 24-h post-MI. GAL-3 is colocalized with cardiomyocytes and endothelial cells in the ischemic area of the LV. GAL-3 is also colocalized with hypoxia-inducible factor-1 alpha (HIF-1α).
Conclusions: We show for the first time that GAL-3 is increased at both transcriptional and translational levels in the LV in early ischemic period, which can possibly be a part of the prosurvival gene expression profile transcribed by HIF-1α. This is significant because it can help in understanding the mechanism of very early response of the myocardium following acute infarction and help devise ways to save the viable tissue before permanent damage sets in.

1. Introduction

Galectins are an ancient family of lectins. They are characterized by evolutionary conserved amino acid sequences and β-galactoside recognition and binding. There are around 15 members of this family known and are found to be widely distributed from lower invertebrates to mammals [1–4]. Galectin-3 (GAL-3) is ~35-kDa protein. It is a unique chimera-like galectin that has one C-terminal carbohydrate recognition domain connected to a long N-terminal domain [5]. It is found on the cell surface and within the extracellular matrix, as well as in the cytoplasm and the nucleus of cells. Its localization depends on factors such as cell type and proliferation state [6–10], cultivation conditions [11], and neoplastic progression [12–16] and transformation [17,18]. The distribution in many types of cells, together with varied subcellular localization, indicates that GAL-3 has many different roles in normal and pathophysiological conditions [19,20].

Intracellular GAL-3 has been suggested to be involved in mitosis, proliferation of cells, and antiapoptotic mechanisms [21–24]. It affects K-Ras [25,26] and Akt proteins [27,28], and so it also regulates differentiation, survival, and death [29,30]. Intranuclear localization of GAL-3 is well documented even in the absence of a nuclear localization signal. It is involved in spliceosome assembly [31] and pre-mRNA splicing [31–33]. Also, it is implicated in regulation of gene transcription [22] and Wnt/β-catenin signaling pathway. Extracellular GAL-3 mediates cell–cell adhesion, cell–matrix interaction, and signaling [34–37] and can induce apoptosis [38].

GAL-3 is expressed in a variety of cells, e.g., endothelial and epithelial cells; activated macrophages [39–41]; activated microglial cells [42,43]; inflammatory cells including macrophages, basophils, mast cells, eosinophils, and neutrophils [44–48]; and subsets of neurons [49]. In tissues, GAL-3 is expressed in the lung, spleen, stomach, colon, adrenal gland, uterus, ovary, prostate, kidney, heart, cerebrum, pancreas, and liver [50].

Recently published data have established a very strong role of GAL-3 in heart failure [51]. Higher levels were associated with recurrent heart failure and increased risk of death in a number of studies [52–55].

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This has led to its use as a prognostic marker in patients with heart failure. GAL-3 also predicted all-cause death [56] and demonstrated a relationship with future heart failure and rehospitalizations in the general population [57]. Despite its established role in heart failure, GAL-3 has not been studied directly in relation to cardiac ischemia. In other organs, e.g. in kidneys, GAL-3 mRNA increased after ischemic injury in acute renal failure in rats [58]. There was also up-regulated expression of GAL-3 in the ischemic brain following transient middle cerebral artery occlusion in rats and in neonatal hypoxic ischemic brain injury [43,59]. We aim to study the direct effects of ischemia on GAL-3 levels in the heart very early in the course of events following myocardial infarction (MI).

2. Materials and methods

2.1. Animal groups

C57B6/J mice were divided into five groups with the following time points: group I, 20 min post-MI ($n=8$); group II, 30 min post-MI ($n=8$); group III, 60 min post-MI ($n=8$); group IV, 4 h post-MI ($n=8$); and group V, 24 h post-MI ($n=8$). Samples from sham-operated animals, which are our controls (20-min sham: $n=8$, 30-min sham: $n=7$, 60-min sham: $n=7$, 4-h sham: $n=7$, and 24-h sham: $n=8$) were also studied. Samples from nonoperated normal animals (naive $n=7$) were also studied. The Animal Research Ethics Committee of the College of Medicine and Health Sciences, UAE University, has approved all experimental procedures (Protocol No. A12/10). All experiments were performed with the guidelines from the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" published by NIH (NIH Publication No. 85-23, revised 1996), and specific national laws have been observed.

2.2. Murine model of MI

C57B6/J mice (male, age: 12–16 weeks; weight: 20–25 g) were anesthetized by an intraperitoneal injection of a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg). The mice were then intubated by transesophageal illumination using a modified 22-gauge plastic cannula and fixed on the operating pad in the supine position by taping all four extremities. The mice were connected to a mouse ventilator (Harvard Apparatus Minivent Hugo Sachs Electronik) which supplied room air supplemented with 100 % oxygen (tidal volume 0.2 ml/min, rate 120 strokes/min). Rectal temperature was continuously monitored and maintained within 36°C–37°C using a heat pad. The lead II ECG (ADInstrument multichannel recorder interfaced with a computer running Power lab 4/30 data acquisition software) was recorded from needle electrodes inserted subcutaneously. MI was induced in the mice by permanently occluding the left anterior descending coronary artery (LAD), as described earlier [60–62].

Briefly, the chest was opened with a lateral incision at the fourth intercostal space on the left side of the sternum. Next the chest wall was retracted for better visualization of the heart. With minimal manipulation, the pericardial sac was removed and the LAD was visualized with a stereomicroscope (Zeiss STEMI SV8). An 8-0 silk suture was passed under the LAD and ligated 1 mm distal to left atrial appendage. Occlusion was confirmed by observing immediate blanching of the left ventricle (LV) post-ligation. An accompanying ECG recording showed characteristic persistent ST elevation which further confirmed ischemia. The chest wall was closed by approximating the third and fourth ribs with one or two interrupted sutures. The muscles returned back to their original position and the skin closed with 4-0 prolene suture. The animal was gently disconnected from the ventilator, and spontaneous breathing was seen immediately. Postoperative analgesic (butorphanol 2 mg/kg, s/c, 6 hourly) was given at the end of the procedure. Sham-operated mice underwent exactly the same procedure described above, except that the suture passed under the LAD is left open and untied. According to the experimental protocol, mice were sacrificed after induction of MI with desired time of ischemia. The hearts were washed in ice-cold phosphate-buffered saline (PBS), right ventricle and both atria were dissected away, and LV was immediately frozen in liquid nitrogen and later stored in −80°C freezer. Blood was also collected in EDTA vacutainers and centrifuged at 3000 RPM for 15 min. The plasma was collected, alliquoted, and stored at −80°C until further analysis. Heart samples from the same time point following LAD ligation were fixed in 10% buffered formal-saline for 24 h. Heart samples from the respective time points were also stored in *RNA later* for RNA extraction and subsequent real-time polymerase chain reaction (PCR).

2.3. RNA isolation and quantitative reverse transcriptase PCR

The mouse LV heart samples were divided into infarcted and noninfarcted tissue using a dissecting microscope. The tissues were homogenized in TRI Reagent (Ambion), and RNA was isolated by phenol–chloroform method [63]. RNA pellet was resuspended in nuclease-free water and stored at −80°C. RNA was quantified using a NanoDrop spectrophotometer and reverse transcribed using High-Capacity cDNA Reverse Transcription kit (Part #4368814, PN 4374967; Applied Biosystems Inc, Foster City, CA, USA) according to the recommended protocol. cDNA was diluted 1:10 and amplified by real-time PCR using target-specific TaqMan gene expression assays using the 7500 Real-Time PCR system (Applied Biosystems). The reaction mix of 20 μL contained 5 μL of sample, 10 μL of TaqMan Gene Expression Master Mix (Part #4369016), 1 μL of Primer probe mix, and 4 μL of nuclease-free water. Each well was run in duplicate for gene of interest and reference primer 18S rRNA (Applied Biosystems). The primers and probes for GAL-3 (Mm00802901_m1) and 18S rRNA (Mm03928990_g1) were purchased from Applied Biosystems. The PCR reaction was carried out as follows: 95°C for 3 min, followed by 95°C for 15 s, 55°C for 15 s, 72°C for 1 min×40 cycles, then followed by 95°C for 15 s, then 60°C for 15 s, and finally 95°C for 15 s. Data acquisition was done by using 7500 software v2.0.6 (Applied Biosystems). Analysis was carried out using the comparative Ct method. The level of GAL-3 expression was normalized to 18S rRNA, and fold changes were calculated relative to expression in sham-operated LV heart tissue using the formula $2^{-\Delta\Delta Ct}$. Many aspects of Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines were taken into consideration for methods and analysis [64].

2.4. Sample processing for protein extraction

Total protein was extracted from heart samples by homogenizing with lysis buffer and collecting the supernatant after centrifugation. For total cell lysate, the heart LV samples were thawed, weighed, and put in cold lysis buffer containing 50 mM Tris, 300 mM NaCl, 1 mM $MgCl_2$, 3 mM EDTA, 20 mM β-glycerophosphate, 25 mM NaF, 1% Triton X-100, 10% wt/vol glycerol, and protease inhibitor tablet (Roche Complete protease inhibitor cocktail tablets). The hearts were homogenized on ice by a homogenizer (IKA T25 Ultra Turrax). The samples were then centrifuged at 14,000 RPM for 15 min at 4°C, supernatant collected, aliquoted, and stored at −80°C until further analysis. Total protein concentration was determined by BCA protein assay method (Thermo Scientific Pierce BCA Protein Assay Kit).

2.5. Sample processing for histology

Hearts were excised, washed with ice-cold (PBS), blotted with filter paper, and weighed. Each heart was sectioned into coronal slices of 2-mm thickness then cassetted and fixed directly in 10% neutral formalin for 24 h, which was followed by dehydration in increasing concentrations of ethanol, clearing with xylene, and embedding with paraffin. Three-micrometer sections were prepared from paraffin blocks and stained with hematoxylin and eosin (H&E). The stained sections were