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Lack of effect of prolonged treatment with liraglutide on cardiac remodeling in rats after acute myocardial infarction

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ABSTRACT

Following the acute phase of a myocardial infarction, a set of structural and functional changes evolves in the myocardium, collectively referred to as cardiac remodeling. This complex set of processes, including interstitial fibrosis, inflammation, myocyte hypertrophy and apoptosis may progress to heart failure. Analogs of the incretin hormone glucagon-like peptide 1 (GLP-1) have shown some promise as cardioprotective agents. We hypothesized that a long-acting GLP-1 analog liraglutide would ameliorate cardiac remodeling over the course of 4 weeks in a rat model of non-reperfused myocardial infarction. In 134 male Sprague Dawley rats myocardial infarctions were induced by ligation of the left anterior descending coronary artery. Rats were randomized to either subcutaneous injection of placebo or 0.3 mg liraglutide once daily. Cardiac magnetic resonance imaging was performed after 4 weeks. Histology of the infarcted and remote non-infarcted myocardium, selected molecular remodeling markers and mitochondrial respiration in fibers of remote non-infarcted myocardium were analyzed. Left ventricular end diastolic volume increased in the infarcted hearts by 62% (from 0.58 ± 0.03 mL to 0.95 ± 0.07 mL, $P < 0.05$) compared to sham operated hearts and left ventricle ejection fraction decreased by 37% (63 ± 1%–40 ± 3%, $P < 0.05$). Increased interstitial fibrosis and phosphorylation of p38 Mitogen Activated Protein Kinase were observed in the non-infarct regions. Mitochondrial fatty acid oxidation was impaired. Liraglutide did not affect any of these alterations. Four-week treatment with liraglutide did not affect cardiac remodeling following a non-reperfused myocardial infarction, as assessed by cardiac magnetic resonance imaging, histological and molecular analysis and measurements of mitochondrial respiration.

1. Introduction

In the event of an acute myocardial infarction (AMI) the ischemic region of the heart suffers myocyte death and myocyte stunning, causing an immediate decrease in the contractile force of the ventricle. In the subsequent days to weeks a progressive apoptotic cell loss occurs in the non-infarcted (remote) region of the left ventricle (LV) [9]. Collectively termed myocardial remodeling, a range of structural and molecular alterations develop in the myocardium, including inflammation [84], accumulation of excessive collagen in the cardiac interstitium [83] and hypertrophy [55,84]. These myocardial remodeling changes cause a negative spiral of decreasing cardiac function that may progress to heart failure. Studies of post-myocardial remodeling in rats have shown that remodeling is almost complete 4 weeks after the myocardial insult, creating a model of severe heart failure in rats with large myocardial infarctions [9,43,57,68]. Hence, this model is suitable for testing drugs that might ameliorate the adverse remodeling effects.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that

Abbreviation: CMR, cardiac magnetic resonance imaging; EF, ejection fraction; AMI, acute myocardial infarction; GLP-1, glucagon-like peptide-1; GLUT, glucose transporter; BNIP3, Bcl-2 19-kDa protein-interacting protein 3; p38 MAPK, p38 mitogen-activated protein kinases; Bad, Bcl-2 family member; LAD, left anterior descending; LV, left ventricle; EDV, end diastolic volume; ESV, end systolic volume

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modifies insulin-mediated effects and metabolism [18,51]. Liraglutide is a modified GLP-1 analog with plasma albumin binding of 99% [60] and a long half-life [1]. Several studies suggested that GLP-1 and its long-acting analogs, including exenatide and liraglutide, may have cytoprotective properties, as well as a beneficial action on cardiac function. Amelioration of experimental reperfusion injury was originally reported for GLP-1 [7], and was confirmed in similar studies using GLP-1 analogs [53,72,76,85]. Long-term beneficial and survival-improving effects in models of myocardial remodeling, heart failure and obesity-associated cardiac pathology have also been reported for these agents [16,44,53,61,65]. Recently, in patients with non-ST-segment elevation myocardial infarction, liraglutide was found to improve left ventricular function [8], and was suggested to be able to prevent the progression of left ventricle remodeling in diabetic patients with acute myocardial infarction undergoing primary PCI [54].

The purpose of the present study was to examine whether liraglutide treatment could favorably affect functional and molecular changes of adverse cardiac remodeling in a rat model of a non-reperfused myocardial infarction.

2. Materials and methods

One hundred and seventy-nine rats were originally used but some died during intubation, surgery or early post-operative phase. A total of 134 (75%) male Sprague Dawley rats survived surgery and were included to perform this study (7 weeks; ~225–275 g; Taconic, Ry, Denmark). Animals were housed at 21 ± 2 °C in a 12-h light/dark cycle with access to standard rat chow and water. The animals were acclimatized for 5–12 days. The Department of Experimental Medicine, University of Copenhagen, and The Animal Experiments Inspectorate, The Ministry of Justice, Denmark approved the experimental procedures. Rats were weighted before operation, and once daily for the rest of the study period.

2.1. Study outline

Myocardial infarction was elicited by ligation of the left anterior descending coronary artery (LAD) [22]. The surgery was performed in general anesthesia using a mix of oxygen and isoflurane at initial 4%, when proper anesthesia was obtained isoflurane was administered at 2–3%. During open chest surgery, the animals were ventilated using a micro-ventilator (MicroVent 1, Hallowell, Pittsfield, USA) at a respiratory rate of 80, a volume of 8 mL/breath and a positive end-expiratory pressure. During anesthesia, cutting the left 3rd and 4th costa ½ cm lateral to the sternum opened the thoracic cage, avoiding bleeding from the internal epigastric artery. We used a retractor to open up the operation field. A pericardiectomy was performed using a small pair of forceps. Passing a fine PDS II 6.0 needle under LAD just caudal to the left atrial appendage occluded the LAD. The rib cage, the pectoral muscles and the skin were closed separately using a vicryl 4.0 suture. Sham operation was performed similar with the only difference being that the PDS II 6.0 needle was not passed through the heart, but placed in a small portion of the pectoral musculature. The animals were sacrificed after 4 weeks by excision of the heart in a Hypnorm (fentanyl/fluanisone) – Dormicum (midazolam) anesthesia. Rats were weighted before operation, and once daily for the rest of the study period. The tibial length was measured with a digital caliper at the end of the experiment. First injection was commenced the day after surgery. Animals were randomized to sham or infarct surgery treatment before operation was commenced and to liraglutide or placebo the day after surgery through a sealed envelope system. All data analysis was performed blinded to treatment.

2.2. Drugs

All animals received either placebo treatment (0.05 mL PBS buffer) or liraglutide (0.05 mL, not adjusted to body weight, 6 mg/ml liraglutide, Victoza®) once daily for 4 weeks. Liraglutide is a long-acting glucagon-like peptide-1 agonist (GLP-1 agonist) developed for the treatment of type 2 diabetes and is commercially available as Victoza® (Novo Nordisk, Denmark). Liraglutide was given once daily as a subcutaneous injection (0.3 mg) starting the day after the operation. Total (albumin-bound plus free) plasma liraglutide concentrations were measured in 4 animals at 6 h and 24 h post-injection for 4 days, and 24 h post-injection for 24 days. The assay has been described in detail [10]. In short, liraglutide concentrations were determined with a radioimmunoassay specific for the N-terminus of GLP-1 [15]. The assay also measures intact biologically active GLP-1. The endogenous GLP-1 is known to be present in low picomolar concentrations [56], thus contributing negligibly to the liraglutide values reported here.

2.3. Cardiac magnetic resonance

CMR was performed after an average of 27 days (range: 24-27 days) in 8 animals in each group. Animals were selected randomly according to scanner capacity. Scanning was carried out on a 7.0 T scanner (Bruker BioSpec, Bruker Medical, Ettlingen, Germany) with a horizontal bore and a 60-mm transmitter-receiver coil (Bruker Medical). The coil was tuned and matched automatically. The rats were placed in a prone position on a rounded plastic plate for insertion into the bore of the scanner. The rats were anesthetized in a mixture of 30%:70% O_2 and air and 2.5–3.5%$_{vol}$ of sevoflurane. Temperature and breathing frequency were monitored and kept around 36.5–37.0 °C and 40–60/min. A stable body temperature was maintained by warm water heating.

We used an intragate protocol for all images. First, a Tri-pilot multi slice, ScanMode 'PilotScan', with a repetition time of 85 ms, an echo time of 1.5 ms, a field of view 35 mm, 3 slices of 1 mm thickness were performed for all initial images. These images were used both to plan the scan and center the rat in the center of the scanner, to obtain axial, sagittal and coronal images of the rat and from these obtain long axis two chamber view, long axis four chamber view, and a short axis view. Then a stack of single slice IntraGateFLASH (fast gradient echo sequence) cine in real short axis view (perpendicular to the axis of the heart) was obtained, using the following parameters: repetition time 4.8 ms, echo time 2.04 ms, Field-of-view 4.4 mm, matrix size 256 × 256, 1 mm thickness with 1 mm gap, bandwidth: 4566.7 Hz, 150 number of repetitions and flip angle: 10. We obtained 15 phases during the cardiac cycle. Continuous slices were applied from base to apex covering the entire heart. All LV and RV volumes were calculated by manually tracing the endocardial border in all 15 time frames in each short axis slide. End diastolic volume (EDV) and end systolic volume (ESV) were defined as largest and smallest volume, respectively. Ejection fraction was stroke volume divided by the end-diastolic volume. LV and RV volumes were computed from the stack of short axis cine images using dedicated software with semiautomatic edge detection of the endocardial contour (CVI42v. 4.0.1, Circle Cardiovascular Imaging, Calgary, Canada). A single operator performed all CMR analyses. Image analysis was performed blinded to knowledge of treatment. LV mass was obtained at EDV and ESV and calculated as myocardial volume multiplied by the myocardial specific gravity (1.05 g/cm^3). Cardiac output was stroke volume multiplied by heart rate. Myocardial infarct size was determined for every slice as the myocardial portion with significant thinning and akinesia or dyskinesia during systole [49]. Taking the sum of infarct volume and dividing by the sum of the total LV myocardial volume calculated *Relative MI size*.

2.4. Tissue preparation and analysis

2.4.1. Histology

After the sacrifice and heart excision, we quantified infarct scar size and the amount of fibrosis in remote myocardium. The heart was immersion-fixed in 10% formalin, cut in half in the transverse plane,