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Chitosan hydrogels significantly limit left ventricular infarction and remodeling and preserve myocardial contractility

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ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form
2 November 2015
Accepted 11 November 2015
Available online 19 November 2015

Keywords:
Myocardial infarction
Ischemic cardiomyopathy
Left ventricular wall stress
Apoptosis
Left ventricular remodeling
Chitosan
Chitosan hydrogels

ABSTRACT

Background: Left ventricular myocardial infarctions (MIs) consist of a central area of myocardial necrosis that is surrounded by areas of myocardial injury and ischemia. We hypothesized that chitosan hydrogels, when injected around the perimeter of MIs in rats, could decrease left ventricle (LV) wall stress by the Law of LaPlace, and therefore myocardial oxygen requirements, and prevent the ischemic and injured myocardium from becoming necrotic. In this manner, chitosan gels could limit LV infraction size and LV remodeling. Chitosan hydrogels are liquid at 25°C but gel at 37°C.

Methods: Seventy Sprague–Dawley rats with ligation of the left coronary artery were treated with either Dulbecco's Modified Eagle Medium (DMEM) or chitosan hydrogel in DMEM, which was injected around the infarct perimeter. Echocardiograms were obtained before MI and at 2, 4, 8, 12, and 16 wk after MI. Hearts from randomly selected rats were harvested at baseline and at the time of echocardiography for determinations of LV infarct size, remodeling, and histopathology.

Results: Infarct sizes as a percentage of the total ventricular myocardium in the DMEM group averaged 17% *versus* 14% in the chitosan group at 4 wk ($P < 0.05$), 18% *versus* 14% at 8 wk ($P < 0.01$), 19% *versus* 14% at 12 wk ($P < 0.001$), and 20% *versus* 14% at 16 wk ($P < 0.001$). Injection of chitosan into the infarctions produced LV wall thicknesses in the MI border zones that averaged 0.66 cm at 4 wk, which were greater than the LV wall thicknesses in the border zones of rats treated with DMEM, which averaged 0.33 cm ($P < 0.01$). Arteriole densities in the MI border zones were 160/mm^2 in the chitosan group but only 92/mm^2 in the DMEM rats ($P < 0.01$). The left ventricular end-diastolic diameters (LVEDs) in the rats averaged 0.73 cm before MI. After MI, LVED increased in the DMEM rats to 0.84 cm at 2 wk, then 0.89 cm at 4 wk, 0.89 cm at 8 wk, 0.89 m at 12 wk, and 0.87 cm at 16 wk. In contrast, LVED in the chitosan rats were on average 19% smaller in comparison with the DMEM rats ($P < 0.05$) and did not significantly change in comparison with their baseline LVEDs. Left ventricular ejection fraction (LVEF) in the rats averaged 83% before infarctions. In the infarction + DMEM group, the LVEFs significantly decreased after MI and averaged 59.7% at 2 wk, 52.5% at 4 wk, 46.1% at 8 wk, 52.4% at 12 wk, and 53.6% at 16 wk ($P < 0.05$). In the

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http://dx.doi.org/10.1016/j.jss.2015.11.012

infarction + chitosan-treated rats, the LVEFs were greater and averaged 67.8% at 2 wk ($P < 0.02$), 68.9% ($P < 0.02$) at 4 wk, 69% ($P < 0.003$) at 8 wk, 65.2% at 12 wk ($P < 0.05$), and 67% at 16 wk ($P < 0.05$).

Conclusions: Chitosan gel can increase LV myocardial wall thickness, decrease infarct size and LV remodeling, and preserve LV contractility.

1. Introduction

Approximately 650,000 people in the United States experience an acute myocardial infarction (MI) each year, and 300,000 people experience recurrent MIs [1]; 120,000–152,000 people die due to their MIs each year. Acute or recurrent MIs are associated with significant upregulation of myocardial metalloproteinases and degradation of extracellular matrix, myocyte apoptosis and necrosis, decreased myocardial wall thickness, increased wall stress in the injured and ischemic myocardium that surrounds the infarction, and decreased myocardial contraction [2]. As a consequence, the left ventricle (LV) dilates and assumes a more spherical shape. However, dilation of the LV and thinning of the LV myocardial wall further increase myocardial wall stress due to the Law of LaPlace where wall stress is equal to LV pressure times LV radius divided by wall thickness. Increased wall stress increases myocardial oxygen requirements, LV chamber dilation, and wall thinning. Increased myocardial oxygen requirements contribute to injured and ischemic myocardium becoming necrotic myocardium. This process sets up a vicious cycle that ultimately results in expansion of the left ventricular infarction and left heart remodeling and failure [3,4]. Consequently, new treatments for acute MI are necessary that will decrease myocardial wall stress, myocardial oxygen requirements, infarct size and limit or prevent LV remodeling, and the development of LV failure. Current treatment of MIs consists of decreasing LV wall stress by reducing elevated arterial systolic pressures to physiological ranges with arterial vasodilators. In addition, increased LV volume, and therefore LV radius, is decreased by treatment with diuretics and venodilators. To date, little work has been done regarding increasing LV wall thickness in subjects with MIs. In the present investigation, we hypothesized that chitosan hydrogels, when injected around the perimeter of MIs in rats, could decrease LV wall stress by increasing wall thickness and therefore decrease myocardial oxygen requirements, and prevent the ischemic and injured myocardium from becoming necrotic. In this manner, chitosan gels could limit LV infraction size and LV remodeling.

Chitosan is a linear polysaccharide, consisting of D-glucosamine and N-acetyl-D-glucosamine linked by glycosidic bonds that is obtained by treating crustacean shells with sodium hydroxide. Chitosan hydrogels consist of a mixture of chitosan, β-glycerol phosphate, and hydroxyethyl cellulose [5–7]. The hydrogels have been used in dressings of open wounds where the gels accelerate wound healing [8–11]. In addition, chitosan has been successfully used in drug delivery systems and for repair and/or regeneration of skin, bone, cartilage, nerves, liver, and muscle [12]. Chitosan hydrogels are liquid at room temperature but undergo gelation and can form a matrix in body tissues at 37°C [5,6]. Therefore, the hydrogels are amendable to delivery by direct syringe injection or LV catheter injection into the ischemic and injured myocardium. The present report documents our experience with chitosan hydrogels in the treatment of acute MIs.

2. Materials and methods

Male Sprague–Dawley rats, weighing 250–350 g, were housed in a temperature-controlled environment with free access to food and water. On the day of surgery, the rats were anesthetized with isoflurane 5% by inhalation, intubated with polyethylene tubing, and mechanically ventilated (Harvard Apparatus) with oxygen and 3%–5% isoflurane. The rats were then placed in the right lateral decubitus position on a heating pad. A thoracotomy was performed in the fourth left intercostal space, the pericardium was opened, and the left anterior descending (LAD) coronary artery was permanently ligated with 5–0 prolene suture 3 mm below its origin from the aorta. MI in each rat was confirmed after LAD ligation by discoloration and akinesis of the anterior myocardial wall and QRS ST segment elevation on the electrocardiogram 1 h after coronary artery ligation.

Male rats were used in the present experiments rather than female rats to avoid hormonal changes that might affect LV remodeling. Seventy male rats were randomly divided into two groups. The control group consisted of 35 rats in which the LAD coronary artery was permanently ligated and, after 1 h, 0.2–0.5 mL of Dulbecco's Modified Eagle Medium (DMEM) was injected around the perimeter of each MI. The infarct + chitosan group consisted of 35 rats that underwent LAD ligation and after 1 h were given 0.2–0.5 mL of chitosan in DMEM around the perimeter of each infarction. In each group, the tip of the injection needle was bent 90° in order that the needle remained parallel with the myocardial wall. Before injection, the syringe connected to the needle was aspirated to ensure the needle had not entered the LV cavity. The chest wall of each rat was then closed with 3–0 Vicryl then 3–0 Prolene sutures in three separate layers, and the isoflurane anesthesia was discontinued. The rats were then continuously monitored for approximately 1–2 h until alert and ambulatory. Carprofen (5 mg/kg, subcutaneous) was given for analgesia every 12 h for 48 h then as needed after surgery. The University of South Florida/James A. Haley Institutional Review and Animal Use Committees approved all our protocols and procedures.

2.1. Chitosan

Two hundred milligrams of chitosan (Sigma), which was 75%–90% deacetylated, was dissolved in 10 mL of distilled water to