Matrix metalloproteinase inhibitors attenuate endotoxemia induced cardiac dysfunction: A potential role for MMP-9

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Abstract

Enhanced cardiac generation of peroxynitrite contributes to septic cardiomyopathy. Since matrix metalloproteinases (MMPs) are activated in vitro by peroxynitrite, we hypothesized that MMPs may contribute to cardiac mechanical dysfunction in sepsis. Rats were injected (i.p.) with either lipopolysaccharide (LPS, 4 mg/kg) or vehicle. MMP inhibitors, either Ro 31-9790 (20 mg/kg), doxycycline (4 mg/kg), or vehicle were administered i.p. 30 min after LPS. At 6 h, when the symptoms of endotoxia peak, hearts were excised and perfused as working hearts with Krebs-Henseleit buffer at 37°C. Cardiac work (cardiac output x peak systolic pressure product) was measured. Perfusate and ventricle samples were analyzed by gelatin zymography to quantify MMP activity.

Cardiac function was significantly depressed in LPS-treated rats compared to control rats (control: 55 ± 4, LPS: 26 ± 6 mmHg*mL*min⁻¹). LPS also caused a loss of 72 kDa MMP-2 activity in the ventricles and the perfusate. Although MMP-9 activity was not detected in the ventricles, LPS resulted in an increase in perfusate 92 kDa MMP-9 activity. The MMP inhibitors significantly improved cardiac function of LPS-treated rats (Ro 31-9790: 38 ± 3, doxycycline: 51 ± 3 mmHg*mL*min⁻¹), had no effect on the loss of MMP-2 activity, and significantly reduced the MMP-9 activity in the perfusate. These results demonstrate, for the first time, that LPS induced cardiac dysfunction is associated with a loss in ventricular MMP-2 activity and the release of MMP-9 from the heart. MMP inhibitors can significantly preserve cardiac mechanical function during septic shock.

Key words: endotoxemia, matrix metalloproteinases, myocardium, plasma

Introduction

Septic shock is a condition caused by massive microbial infection and is characterized by hypotension and cardiac dysfunction (septic cardiomyopathy) [1]. Septic patients have severely reduced cardiac ejection fractions and depressed measures of the Frank-Starling relationship [2]. A large body of evidence has suggested that this cardiac dysfunction is caused by pro-inflammatory cytokines which are elevated during sepsis and which enhance myocardial production of nitric oxide and superoxide [3]. These two molecules combine to form the highly toxic oxidant peroxynitrite at a diffusion-limited rate [4]. Peroxynitrite exerts its cytotoxic effects through the nitration of free tyrosine residues and oxidation of sulfhydryl moieties. Evidence of peroxynitrite formation has been found in the heart [5] and lungs [6] of septic patients, and in the heart [7] and plasma of endotoxemic rats [8]. Despite these advances in our understanding of septic shock, mortality remains high. Thus, new factors and potential targets for therapeutics need to be sought out. We speculate that matrix metalloproteinases (MMPs) are potential mediators of sepsis-related pathologies.

MMPs are a large family of zinc dependent endopeptidases which have been recognized for their ability to degrade components of the extracellular matrix. Increased MMP activity has been associated with a wide variety of long-term cardio-
vascular pathologies, including heart failure and atherosclerosis [9, 10]. Recently, we have demonstrated that MMP-2 activation and release mediates acute cardiac failure following ischemia-reperfusion injury through the cleavage of troponin I [11, 12]. The latter study and a number of other investigations have demonstrated that MMPs can act acutely on a variety of non-extracellular matrix substrates [13–15].

Interestingly, peroxynitrite activates MMPs in a concentration-dependent manner in vitro [16]. Thus, MMPs may be activated in the septic heart through an increased production of peroxynitrite and ultimately contribute to acute cardiac dysfunction. In order to examine the potential role of MMPs in septic cardiomyopathy, we used a well established rodent model of endotoxemia and coadministered MMP inhibitors. At the height of endotoxic symptoms the hearts were excised and perfused ex vivo in order to assess cardiac mechanical function and MMP activities in both the ventricles and the perfusate.

Materials and methods

This investigation conforms to the Guide to the Care and Use of Laboratory Animals published by the Canadian Council on Animal Care (revised 1993).

Rat model of endotoxemia and isolated heart perfusion

Male Sprague-Dawley rats (250–300 g) were given either a bolus intraperitoneal injection of a non-lethal dose of lipo-polysaccharide (LPS; Salmonella typhosa 0901, Difco, 4 mg/kg) or pyrogen-free water (control, n = 7). 0.5 h later, rats were administered i.p. with the MMP inhibitors Roche 31-9790 (20 mg/kg, n = 10), or doxycycline (4 mg/kg, n = 6), or a volume matched amount of their respective vehicles (polyethylene glycol, n = 6, or pyrogen-free water, n = 10).

Six hours post LPS injection, when the symptoms of endotoxia peak in this model [7, 17], animals were sacrificed by sodium pentobarbital overdose (100 mg/kg, i.p.). Hearts were rapidly excised and perfused as working hearts with 110 mL of recirulating Krebs-Henseleit buffer containing 11 mM glucose, 5 mM pyruvate, 100 µU/mL insulin, 1.75 mM Ca²⁺, 0.5 mM EDTA, and 0.1% bovine serum albumin [18, 19]. This buffer was delivered from the oxygenator (supplied with 95% O₂/5% CO₂) into the left atrium at a preload hydrostatic pressure equivalent to 9.5 mmHg. The perfusate was then ejected by the heart into a compliance chamber (containing 1 mL of air) and into the aortic outflow line. The hydrostatic afterload pressure was set at 70 mmHg.

Heart rate and peak systolic pressure were measured by a Gould P21 pressure transducer in the aortic flow line. Aortic flow and cardiac output were monitored using Transonic flow probes in the afterload and preload lines respectively. Cardiac work, the product of cardiac output (mL·min⁻¹) and peak systolic pressure (mmHg), was noted after 20 min of equilibration in working mode. At this time, 2 mL of perfusate was collected and stored for future processing. The ventricles were snap frozen in liquid nitrogen and stored at –80°C for later processing.

Preparation of ventricular homogenates

Frozen ventricular tissue was crushed by mortar and pestle cooled to the temperature of liquid nitrogen. The resulting powder was diluted 1:4 w/v in 50 mM Tris-HCl (pH 7.4) buffer containing 3.1 mM sucrose, 1 mM dithiotheireitol, 10 µg/mL leupeptin, 10 µg/mL soybean trypsin inhibitor, 2 µg/mL aprotenin and 0.1% Triton X-100. This solution was then homogenized with an Ultra-Turrex disperser using four strokes of 4 sec duration. The homogenate was centrifuged at 10,000 g for 5 min at 4°C and the supernatant was kept on ice for immediate assay of MMP activities.

Ventricular homogenate and perfusate protein contents were determined by the bicinchoninic acid method (BCA kit, Sigma), using bovine serum albumin as a standard.

Measurement of MMP activity by zymography

Gelatinolytic activities of MMPs were examined as previously described [20]. Eight percent polyacrylamide gels copolymerized with gelatin (2 mg/mL, type A from porcine skin, Sigma) were prepared. Non-heated samples were diluted with water in order to load a constant amount of protein per lane (perfusate, 15 µg; ventricular homogenate, 40 µg). A standard was loaded into each gel (supernatant of phorbol ester activated HT-1080 cells, American Type Culture Collection) in order to normalize activities between gels. Following 1.5 h of electrophoresis, the gels were washed with 2.5% Triton X-100 for 1 h at room temperature (with three changes of solution) to remove sodium dodecyl sulphate. Gels were then incubated for 38–48 h at 37°C in incubation buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM CaCl₂, and 0.05% NaN₃). After incubation the gels were stained with 0.05% Coomassie Brilliant Blue (G-250, Sigma) in a mixture of methanol:acetic acid:water (2.5:1:6.5, v/v) and destained in aqueous 4% methanol:8% acetic acid (v/v). Gelatinolytic activities were detected as transparent bands against the dark blue background. All gelatinolytic activities reported could be inhibited by addition of the matrix metalloproteinase inhibitor o-phenanthroline (100 µM) to the incubation buffer. Zymograms were digitally scanned and band intensities were quantified using SigmaGel software (Jandel Corporation).