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Beta blocker metoprolol protects against contractile dysfunction in rats after coronary microembolization by regulating expression of myocardial inflammatory cytokines

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ABSTRACT

Aims: To examine the effects of metoprolol on expression of myocardial inflammatory cytokines and myocardial function in rats following coronary microembolization (CME).
Main methods: Male rats were randomly assigned to receive either sham-operation (control group), CME plus saline (CME group), or CME plus metoprolol (metoprolol group). CME was induced by injecting 3000 polyethylene microspheres (42 μm) into the left ventricle during a 10-second occlusion of the ascending aorta. Metoprolol (2.5 mg/kg) or saline was administered as three intravenous bolus injections after CME. At 3 h, 6 h, 12 h, 24 h and 4 weeks after CME, myocardial function was measured with echocardiography; and the mRNA and protein levels of tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10) and interleukin 1-β (IL-1β) were determined.
Key findings: Induced CME led to markedly higher mRNA and protein levels of TNF-α, IL-1β and IL-10 at 3, 6, 12, and 24 h, as well as reduced left ventricular function, compared to the control group. Metoprolol administration reduced TNF-α and IL-1β levels, but increased IL-10 levels at 3, 6, 12, and 24 h compared to the CME group. Moreover, metoprolol treatment resulted in significantly improved left ventricular function at 12 h, 24 h and 4 weeks, but afforded no cardiac protection at 3 h and 12 h, compared to the CME group.
Significance: Higher levels of TNF-α and IL-1β in rats following CME are associated with the development of myocardial contractile dysfunction. Metoprolol-conferred protection against progressive contractile dysfunction following CME may be mediated by its anti-inflammation potential.

Introduction

Coronary microembolization (CME) is a spontaneous event caused by atherosclerotic plaque rupture in patients with acute coronary syndromes and a potential iatrogenic complication in patients undergoing thrombolytic therapy and coronary interventions (Morishima et al., 2000). Experiments on animals have shown that CME can induce a pronounced inflammatory reaction that is associated with progressive contractile dysfunction (Dörge et al., 2000). Tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine, is shown to play an important role in triggering an inflammatory cascade and mediating progressive contractile dysfunction after CME (Dörge et al., 2002). Also, previous studies have demonstrated that interleukin 1-β (IL-1β) serves as an effector cytokine, impairing myocardial contraction in rat models and mediating toxic effects on cultured cardiomyocytes (Schulz et al., 1995; McTiernan et al., 1997). Interleukin-10 (IL-10) is a pleiotropic immunomodulatory cytokine that functions at different levels of the immune response (D'Orazio and Niederkorn, 1998). IL-10 can act as a potent anti-inflammatory factor against some of the deleterious effects of pro-inflammatory cytokines (Damas et al., 2001).

Adrenergic activation contributes to increased myocardial expression of TNF-α and IL-1β in an experimental model of heart failure. Attenuation of TNF-α and IL-1β expression has been linked to the beneficial effects of β-adrenergic blockade (Wang et al., 2003). Clinical studies have shown that intracoronary β-adrenergic blocker administration before coronary interventions reduces complications caused by CME (Prabhu et al., 2000). However, little is known about the effects of metoprolol treatment on the pro- and anti-inflammatory cytokines and cardiac function following CME. In this study, we tested the hypothesis that the β-adrenergic blocker metoprolol can ameliorate myocardial dysfunction by inhibiting pro-inflammatory cytokines and increasing anti-inflammatory cytokines in a rat CME model.

Materials and methods

Animals

One hundred and fifty male Sprague Dawley (SD) rats 12–14 weeks old and weighing 200–250 g were maintained at the Experimental

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Animal Center of Guangxi Medical University. The rats were housed under conditions of 23 ± 1 °C temperature, 40 ± 5% humidity, and 12 h/12 h light/dark cycle, with free access to standard rat food and tap water. The animal experiments were performed in accordance with the European Community Guidelines for Care and Use of Animals, and approved by the Ethic Committee for Animal Use of Guangxi Medical University.

Study protocol and experimental preparations

The 150 male SD rats were randomly assigned to receive either sham-operation (control group, $n = 50$), CME induction (CME group, $n = 50$), and CME induction plus metoprolol treatment (metoprolol group, $n = 50$). CME was experimentally induced in rats as previously described (Kalenikova et al., 1998). In brief, after general anesthesia was induced with sodium pentobarbital at a dose of 30–40 mg/kg, the trachea was intubated through a midline cervical incision and a respirator was connected. A left lateral thoracotomy was performed through the fourth intercostal space, and the pericardium was opened. Next, 0.1 mL of a suspension containing 3000 microspheres (42 μm diameter Dynospheres, Dyno Particles, Lillestrøm, Norway) in saline solution was injected into the left ventricle during a 10-second occlusion of the ascending aorta with a vascular clamp. After injection of the microspheres, the vascular clamp was removed. Rats in the sham-operated control group were given normal saline without microspheres. Rats in the metoprolol group were intravenously administered three bolus injections of metoprolol (2.5 mg/kg) at approximately 5-minute intervals at 30 min following CME induction (Chen et al., 2005) .The rats were sacrificed at 3 h, 6 h, 12 h, 24 h, and 4 weeks after operation (n = 10 at each time point in each group).

Echocardiography

Rats were lightly anesthetized with an intraperitoneal injection of sodium pentobarbital (30–40 mg/kg). Left parasternal and left apical echocardiographic images of anesthetized rats lying in a supine position were obtained using the Hewlett Packard Sonos 7500 ultrasound instrument (Philips Technologies, USA) equipped with a 10.0 MHz transducer. A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles. Left ventricular M-mode tracings were used to define the internal systolic and diastolic diameters. Left ventricular volume was calculated according to the formula $V = 1.04 \times D^3$, in which D is the left ventricular internal diameter. Fractional shortening (FS) is defined as $[(LVIDd + LVIDs)/LVIDd] \times 100\%$, in which LVEDd is the left ventricular end-diastolic diameter and LVEDs is the corresponding left ventricular end-systolic diameter. Left ventricular ejection fraction (LVEF) is defined as $[(LVEDV + LVESV)/LVEDV] \times 100\%$, in which LVEDV is the left ventricular end-diastolic volume and LVESV is the left ventricular end-systolic volume. Measurements represent the mean of at least three consecutive cardiac cycles (Litwin et al., 1995).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from 100 mg heart tissue using the Trizol reagent kit (Invitrogen). Reverse transcription was performed with All-in-One™ First-Strand cDNA Synthesis Kit (GeneCopeia, USA) according to the manufacturer's instructions. qRT-PCR for gene expression analysis was performed on an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems, USA). The primer sets for qRT-PCR amplification were 5′-GCCCAGACCCTCACACTC-3′ and 5′-CCACTCCAGCTGCTCCTCT-3′ for TNF-α; 5′-TGTGATGAAAGACGGCACAC-3′ and 5′-CTTCTTCTTTCGGGTATTGTTTGG-3′ for IL-1β; and 5′-GAACCACCCGGCGTCTAC-3′ and 5′-AGGGATGAGGGCAAGTGAAA-3′ for IL-10. β-actin was used as an interval control, and the corresponding DNA was amplified using 5′-CCCATCTATGAGGGTTACGC-3′ as the forward primer and 5′-TTTAATGTCACGCACGATTTC-3′ as the reverse primer. qRT-PCR was carried out in a 20 μL reaction system containing 10 μL All-in-One™ qPCR Mix, 2.0 μL each primer (2 μM), 2 μL cDNA, and 4 μL ddH_2O. qRT-PCR was performed with the following protocol: (1) an initial predenaturation step at 95 °C for 10 min; (2) 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, and extension at 72 °C for 15 s. Fluorescence data were collected at the end of each cycle. Relative gene expression levels of the three cytokines were calculated using the ΔΔCt-method.

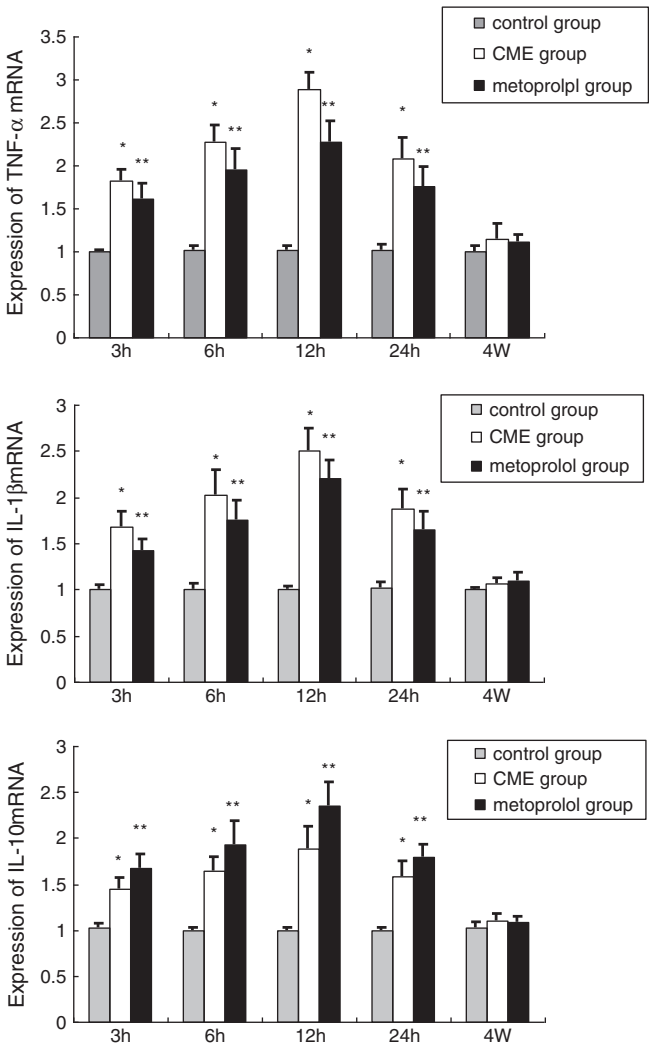

Fig. 1. The mRNA levels of TNF-α, IL-1β, and IL-10 in rat myocardial cells as detected by qRT-PCR. Comparison of TNF-α, IL-1β, and IL-10 mRNA levels among the three groups at various time points. $^{*}P<0.05$, compared to control group at the same time points; $^{**}P<0.05$, compared to CME group at the same time points.

Histological examination and immunohistochemistry

After echocardiography was performed, the hearts were arrested in diastole by injecting 2 mL of 10% KCl into the tail vein of the rats.

Fig. 2. Analysis of immunohistochemistry results. A: Protein expression of TNF-α, IL-1β, and IL-10 in rat myocardial cells detected 12 h after CME (×400). B: Comparison of TNF-α, IL-1β, and IL-10 protein levels among the three groups at various time points. $^{*}P<0.05$, compared to control group at the same time points; $^{**}P<0.05$, compared to CME group at the same time points.