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Propofol attenuates myocardial ischemia reperfusion injury partly through inhibition of resident cardiac mast cell activation

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ABSTRACT

Cardiac mast cell activation is involved in the process of myocardial ischemia reperfusion (I/R) injury and exacerbates myocardial infarction. Propofol, an anesthetic with antioxidant property, can reduce myocardial infarct size in I/R injury. The present study was designed to investigate whether propofol can attenuate myocardial I/R injury by inhibiting resident cardiac mast cell activation by a Langendorff model. Thirty rats were randomly assigned to 5 groups ($n = 6$ per group): control group and four test groups (I/R, I/R + compound 48/80, I/R + propofol, I/R + compound 48/80 + propofol). Cultured RBL-2H3 cells were pretreated with propofol and subjected to mast cell degranulator compound48/80 (C48/80).Microscopically, degradation of myofibrillar and degranulation of mast cells were studied using hematoxylin-eosin toluidine blue staining techniques. After the effluent was assayed for tryptase, LDH, CK-MB and cTnI, myocardial tissue was evaluated for cytokine levels and infarct area. Heart subjected to I/R showed significantly increased expression of cytokines (TNF-α and IL-6), LDH, CK-MB and cTnI. In addition, the I/R-induced heart also showed greater histopathological injury and a larger infarction zone, following increased mast cell degranulation with concomitant rise in tryptase. Mast cell degranulation by C48/80 further aggravated I/R injury. However, all of these effects were suppressed by propofol pretreatment, which also abrogated C48/80-mediated exacerbation of I/R injury. Also, propofol attenuated the C48/80-evoked tryptase and histamine release in RBL-2H3 cells. It is concluded that pretreatment of propofol confers protection against I/R injury partly by inhibiting resident cardiac mast cell activation.

1. Introduction

Acute myocardial infarction (AMI) resulting from a prolonged myocardial ischemia without reperfusion is a chief cause of morbidity and mortality worldwide [1]. Ischemia of the heart initiates a complex and interrelated sequence of events leading to myocardial dysfunction and damage [2]. The immediate restoration of blood supply has protective effects on the ischemic heart but can itself also induce cardiomyocyte death, known as myocardial reperfusion injury [3,4]. Both processes adversely affect the cardiac performance, treatment, and prognosis of patients with AMI. It is well known that immune and inflammatory pathways initiated by the innate immune system are implicated in the whole process of myocardial ischemia–reperfusion (I/R) injury [5,6]. Mast cells are multifunctional immune cells best known for their role in bothersome symptoms (e.g., itchy skin, swelling of the eyes, and runny nose), immediate hypersensitivity, and chronic allergic reactions [7,8]. In cardiovascular tissue, they are mainly located in the vicinity of small blood vessels and produce vasoactive mediators including cytokines, rennin, histamine and proteolytic enzymes.

Cardiac mast cells have been shown to be associated with congestive heart failure [9], dilated cardiomyopathy, hypertension, mitral regurgitation, and myocardial infarction [10,11]. An increased number of degranulated mast cells was observed on the adventitia backing ruptured plaques in a series of 17 autopsied myocardial infarction patients [12]. Gilles and colleagues determined that TNF-α was mainly released by exocytosis from cardiac mast cells in the first few hours during I/R of canine heart [13]. Also, an abundance of histamine and tryptase in mast cells was associated with immunologic injury in the failing and ischemic heart [14]. Furthermore, Jaggi and colleagues reported that ketotifen and low-dose carvedilol, which are usually used as mast cell stabilizers, could profoundly attenuate myocardial I/R injury [15].

Propofol (2,6-diisopropylphenol) is an intravenous sedative-hypnotic agent that has been widely used in clinical anesthesia and intensive care units for its rapid access to cellular and subcellular

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membrane compartments. Previous work has elucidated that propofol can attenuate post-ischemic myocardial cellular injury and oxidative stress in patients undergoing cardiac surgery and can protect the heart from serious ventricular arrhythmias during acute coronary occlusion [16,17]. Other studies also demonstrated that propofol can enhance mitochondrial antioxidant activity, leading to reduced cardiac injury in an isolated ischemic rat heart model, and that propofol exerted its cardioprotective effect by increasing red cell and cardiac tissue antioxidant capacity both in vitro and in vivo [18,19]. But the mechanism by which propofol reduces myocardial I/R injury has not been clearly elucidated. Propofol also exerts dose-dependent inhibition of mast cell exocytosis and chemotaxis in vitro [20]. A recent study showed that propofol could inhibit NADPH oxidase-mediated mast cell activation, resulting in attenuation of both the small intestinal I/R injury itself and resultant lung injury [21,22]. However, it is still unclear whether the protective effects of propofol in myocardial I/R injury are related to mast cell activation. In our study, compound 48/80, a secretagogue for mast cells that can release histamine and several proteases, was used as a tool to induce mast cell activation, with the objective of assessing whether propofol could suppress mast cell activation.

2. Materials and methods

2.1. Cell culture

RBL-2H3 cells (rat basophilic leukemia cell line) were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China).RBL-2H3 cells were grown in minimum essential medium (MEM; Gibco), supplemented with 100 U/ml of penicillin (Gibco), 100 g/ml of streptomycin (Gibco), and 10% fetal bovine serum (Gibco) at 37 °C in 5% CO_2 with 95% humidity.

2.2. Assay of tryptase and histamine in RBL-2H3

RBL-2H3 cells were pretreated with propofol (2,6-diisopropylphenol,50 μmol/L, Sigma, USA) for 15 min before C48/80 (10 μg/ml, Sigma, USA) stimulation for 30 min. The released tryptase and histamine were separated from the cells by centrifugation at 2000 rpm for 10 min at 4 °C. 100 μl of supernatants were mixed with 100ul of 0.8 mmol/lα-*N*-benzoyl-L-arginine-p-nitroanilide (BAPNA, Sigma, USA) in Tris buffer and incubated for up to 72 h at 37 °C. All samples and standards were run in duplicate. The appearance of nitroaniline was then measured at 405 nm in a spectrophotometer. In addition, the measurement of histamine in the supernatant was measured by a commercial ELISA kit (H171; NanJing Jiancheng Bioengineering Institute, Nanjing, China).

2.3. Animals

Wister rats (10 weeks old, 180–240 g) were purchased from the Shandong University Experimental Animal Center (Jinan, China) and they were raised in different cohort. All rats were housed in a room maintained at 22 °C and 45%–55% humidity. Sterile water and standard chow diet were available ad libitum throughout the entire feeding period. All rat experiments were performed in accordance with the principles and guidelines of the Care and Use of Laboratory Animals of Shandong University, and all the procedures were approved by the Medical Ethics Committee for Experimental Animals, Shandong University, China (number ECAESDUSM 2012029).

2.4. Isolated perfused mouse hearts

Metabolic and cellular responses to I/R injury were assessed using isolated, perfused rat heart. All rats were allowed to adapt for at least three days before experiments. After intraperitoneal injection of sodium pentobarbital (200 mg/kg) and median thoracotomy, hearts were quickly excised and immersed in ice-cold oxygenated Krebs–Henseleit solution (KHs) composed of (in mM) NaCl 113, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 1.2, glucose 11 to minimize ischemic damage arising from the isolation protocol, and then immediately placed in a modified Langendorff system via an aortic cannula for perfusion with KHs gassed with 95% O_2, 5% CO_2 (pH 7.4, 37 °C) with a constant flow rate of 10 ml/min. Elapsed time between isolation of the heart and cannulation was approximately 1 min. Spontaneously beating hearts were perfused during a 15-minute stabilization period and made globally ischemic for 30 min by terminating the coronary flow, followed by 60 min of reperfusion. Samples of coronary effluent perfusate (500 μl) were assayed for LDH, CK-MB, cTnI and tryptase analysis, and the hearts were then rapidly excised and stored at − 80 °C until further analysis.

2.5. Experimental protocol

Hearts were randomly divided into five groups (n = 6 per group): (1) the control group (Group Control), which had constant perfusion with KHs for 120 min; (2) Group IR, which had 30 min of stabilization and 30 min of global ischemia followed by 60 min of reperfusion; (3) Group IR + P, which had 15 min of propofol (50 μmol/L, Sigma, USA) [23] after 30 min of equilibration; (4) Group C48/80, which had three immediately consecutive bolus injections of C48/80 (20 μg, Sigma, USA) prior 30 min of global ischemia [24], (5) Group C48/80 + P had a pretreatment of propofol (50 μmol/L, Sigma, USA) for 15 min and following injection with C48/80 (20 μg, Sigma, USA) before ischemia. Tryptase was assessed before induction of global ischemia (baseline) and at different timepoints during reperfusion: immediately after starting reperfusion, 5 min (R5min), and 60 min (R60min) after starting reperfusion. Coronary effluent perfusate was also collected before global ischemia (baseline), at different timepoints during reperfusion (immediately, 5 min [R5min] and 60 min [R60 min]) and stored at − 20 °C for other biochemical evaluations. And three independent repeats of each experiment were performed in our research.

2.6. Assay for tryptase activity in perfusate

Tryptase is the unique mediator released during mast cell degranulation [25]. During heart perfusion, 50 μl of coronary effluent perfusate was immediately incubated for measurement of tryptase enzymatic activity [26]. After 72 min of incubation with 50 μl of 0.8 mmol/l BAPNAat 37 °C, the absorbance of nitroaniline product was measured colorimetrically at 405 nm with spectrophotometer. All samples and standards were run in duplicate.

2.7. Detection of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and cardiac troponin I (cTnI) levels

Increased LDH, CK-MB and cTnI concentrations were used as indices of myocardial cell injury. The levels of LDH in coronary effluent perfusate were determined using a commercial ELISA assay kit (Jiancheng Bioengineering Institute of Nanjing, China); CK-MB and cTnI concentrations in the transudate were measured by an enzyme-linked immunosorbent assay (Shanghai Langdun biotech company, China). Standards and samples were plated in duplicate and the absorbance was measured at 450 nm using a Varioskan Flash multifunction plate reader (Thermo Scientific).

2.8. Real-time polymerase chain reaction analysis of TNF-α and IL-6

Levels of gene expression were measured by real-time polymerase chain reaction analysis (qPCR). Hearts were blotted dry and stored at − 20 °C, and then total RNA extraction was performed using a commercial kit following the manufacturer's instructions (Aidlab Biotech, Beijing China). The cDNA was synthesized with the SYBR Green PCR kit

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