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Journal of Nutritional Biochemistry 31 (2016) 122–126

Journal of Nutritional Biochemistry

Linoleic acid attenuates cardioprotection induced by resolvin D1

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Received 1 April 2015; received in revised form 23 November 2015; accepted 22 December 2015

Abstract

We previously observed that resolvin D1 (RvD1), a metabolite of the omega-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid, reduces infarct size by a mechanism involving the PI3-K/Akt pathway. In parallel, the beneficial effect of a high omega-3 PUFA diet on infarct size can be attenuated by increased omega-6 PUFA consumption. The present study was designed to determine if augmented linoleic acid (LA), an omega-6 PUFA administered at the same time, attenuates the cardioprotective action of RvD1.

Male Sprague–Dawley rats received 0.1 μg RvD1 alone or with one of three LA doses (1, 5 or 10 μg) directly into the left ventricle chamber 5 min before ischemia. The animals underwent 40 min of ischemia by occlusion of the left descending coronary artery followed by 30 min or 24 h of reperfusion. Infarct size and neutrophil accumulation were evaluated after 24 h of reperfusion, while caspase-3, -8 and -9 and Akt activities were assessed at 30 min of reperfusion.

LA attenuated cardioprotection afforded by RvD1, resulting in significantly increased infarct size. Neutrophil accumulation and Akt activity were similar between groups. Caspase activities, especially caspase-9, which could be activated by ischemia, were stimulated in the presence of LA, suggesting that this omega-6 PUFA accentuates ischemia intensity.

The present results indicate that LA significantly attenuates the beneficial effect of RvD1 on infarct size. Therefore, reduction of omega-6 intake should be considered to maintain the protection afforded by RvD1.

Keywords: Myocardial infarction; Omega-3; Resolvin D1; Omega-6; Linoleic acid

1. Introduction

Resolvins (Rv) are metabolites derived from long-chain omega-3 polyunsaturated fatty acids (PUFAs). They could limit myocardial damage when given at the time of infarction [1–3] and even during the reperfusion period [2,4]. Series E Rv (RvE) are derived from the omega-3 PUFA eicosapentaenoic acid, whereas series D Rv (RvD) come from the omega-3 PUFA docosahexaenoic acid (DHA) [5,6], and both interact with G-protein-coupled receptors [7–10]. It has recently been suggested that these metabolites induce cardioprotection by activating signaling pathways, such as PI3-K/Akt, and by limiting inflammation [1–3,11].

The diet in industrialized countries is poor in omega-3 PUFA, whereas consumption of omega-6 PUFA is disproportionally large. The actual ratio of omega-6/-3 in these countries is 15–20:1 [12,13]. Omega-6 and omega-3 PUFAs are both metabolized by cyclooxygenase and lipoxygenase, which results in competition of the two substrates for these enzymes [14]. Large amounts of omega-6 PUFAs promote inflammatory responses compared with omega-3 PUFA s[15], with undesirable effects during myocardial infarction (MI). For instance, augmentation of the omega-6/-3 PUFA ratio in the diet results in larger infarct size as observed in our experimental model [16]. Competition between omega-3 and omega-6 PUFAs is thus important in determining the final outcome.

With that in mind, it is important to determine if the cardioprotection afforded by Rv can be attenuated by omega-6 PUFAs. Linoleic acid (LA), an omega-6 PUFA found in most vegetable oils [17], can induce vasoconstriction in coronary arteries [18] and can be converted to arachidonic acid (AA), the precursor of many inflammatory molecules, such as prostaglandins and leukotrienes [14].

The objective of the present study is to demonstrate if LA can inhibit the cardioprotective effect of RvD1, an omega-3 PUFA metabolite.

2. Methods

A total of 74 male 3-month-old Sprague–Dawley rats (Charles River Canada, St-Constant, QC, Canada), weighing 325–350 g at the start of the experiments, were handled in compliance with regulations of the local Animal Care Committee and *Guidelines of the Canadian Council on Animal Care*. They were housed individually under constant conditions (21°C–22°C temperature and 40%–50% humidity), including a 12-h dark–light cycle beginning at 08:00 am. Chow pellets and tap water were available *ad libitum* throughout the study. The rats were randomly

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http://dx.doi.org/10.1016/j.jnutbio.2015.12.016
0955-2863/

assigned to one of four groups after an acclimatization period of 3 days postdelivery by the supplier: 0.1 μg RvD1 with vehicle (0.9% NaCl) or 0.1 μg RvD1 combined with one of three LA doses: 1.0, 5.0 or 10 μg, delivered directly into the left ventricle (LV) cavity 5 min before ischemia. RvD1 and LA were obtained from Cayman Chemical (Ann Arbor, MI, USA) and dissolved in 0.5 ml NaCl 0.9%. The animals were sacrificed after 24 h of reperfusion ($n=8$–9/group) to measure infarct size and neutrophil activity. Two rats died in the first minute of reperfusion and were excluded from the study (one in the 1.0 μg LA + RvD1 group and the other in the 5.0 μg LA + RvD1 group). A subgroup of rats was sacrificed 30 min after the onset of reperfusion ($n=9$/group) for biochemical analysis (caspase-3, -8 and -9 and Akt activities) and hemodynamic data.

2.1. Protocol 2

The rats were randomly assigned to one of two groups after an acclimatization period of 3 days postdelivery by the supplier: 0.1 μg RvD1 with vehicle (0.9% NaCl) administered 5 min before ischemia in combination with vehicle (saline) or 10 μg of linoleic acid given at the onset of reperfusion. The animals were sacrificed after 24 h of reperfusion ($n=4$/group) to measure infarct size.

2.2. Surgical procedure

Anesthesia was induced by intraperitoneal ketamine/xylazine injection (60 and 10 mg/kg, respectively). Subsequently, the rats were intubated, and anesthesia was maintained under isoflurane (1%–2%) ventilation. Electrocardiograms and heart rate (HR) were monitored throughout the procedure. Left thoracotomy at the fifth intercostal space enabled occlusion of the left anterior descending coronary artery with 4-0 silk suture (Syneture; Covidien, Mansfield, MA, USA) and plastic snare, with confirmation by ST-segment alterations and the presence of ventricular subepicardial cyanosis. The suture was removed after 40 min of ischemia, permitting myocardial tissue reperfusion. The rats were sacrificed after 30-min or 24-h reperfusion. In animals submitted to 24-h reperfusion, the thorax was closed with 2-0, 3-0 and 4-0 silk sutures (Syneture; Covidien), and the animals were injected subcutaneously with 15,000 IU penicillin G (Duplocillin LA; Intervet Canada Ltd., Whitby, ON, Canada) for antibiotic prophylaxis and 2 mg/kg buprenorphine for analgesia before being returned to their respective cages.

2.3. Hemodynamic data

HR and mean arterial pressure (MAP) were measured by the tail cuff technique (Kent Scientific Corporation, Torrington, CT, USA) before occlusion and at 10 min of ischemia.

2.4. Measurement of infarct size

After the reperfusion period, the rats were restrained in a cone bag and rapidly decapitated. In animals with 24-h reperfusion, their hearts were immediately removed, placed in a dish kept on crushed ice and washed with saline by retrograde perfusion into the aorta. The left anterior descending coronary artery was occluded at the same site as for MI induction (see above) to map the area at risk (AR) by Evans blue infusion (0.5%). The hearts were frozen (−80°C for 5 min), sliced into four transverse 2-mm sections and placed in 2,3,5-triphenyltetrazolium chloride solution (1%, pH 7.4) at 37°C for 10 min to better distinguish necrosis, the infarct size (I), from the AR. The different regions were carefully drawn on a glass plate, photocopied and cut. Thereafter, the complete infarct region, AR and LV were weighed separately to express MI as percentage of I of the AR [(I/AR)×100] and AR as percentage of the LV area [(AR/LV)×100]. In animals with 30-min reperfusion, hearts were cut into four to five transversal slices and prepared for analysis. The area at risk was divided into two halves: the lower half was the endocardial region, and the upper part was the epicardial region. Samples were kept at −80°C until needed for biochemical analysis.

2.5. Biochemical analysis

2.5.1. Caspase-3, -8 and -9 activities

Caspase-3, -8 and -9 activities were measured according to a previously described protocol [19]. Tissues were homogenized by sonication in lysis buffer and incubated for 30 min on ice. The tissue homogenates were centrifuged at 4°C for 10 min. Enzymatic reactions were undertaken in reaction buffer with 25 mg of protein (40 μM) (attested by the Bradford method) and fluorescent substrate (Ac-DEVD-AMC for caspase-3, Ac-IETD-AMC for caspase-8 and Ac-LEHD-AMC for caspase-9). Reactions were studied after incubation in the dark for 3 h at 37°C and stopped with the addition of 0.4 M NaOH and 0.4 M glycine buffer. Fluorescence was quantified by spectrofluorometry (Photon Technology International, Lawrenceville, NJ, USA) at excitation wavelengths of 365 nm for caspase-3 and -8 and 315 nm for caspase-9 and at emission wavelengths of 465, 430 and 435 nm for caspase-3, -8 and -9 activities, respectively.

2.5.2. Western blotting of Akt

Tissues were homogenized by sonication in lysis buffer [1% Triton X-100, 0.32 mol/L sucrose, 10 mmol/L Tris (pH 8.0), 5 mmol/L EDTA, 2 mmol/L DTT, 1 mmol/L PMSF, 10 mg/mL leupeptin, 10 mg/mL Pepstatin A and 10 mg/mL aprotinin]. The tissue homogenates were incubated for 30 min at 4°C and centrifuged at 10,000*g* for 15 min. Protein concentrations of the supernatant were quantified by the Lowry method. Aliquots of 100 mg protein were loaded in polyacrylamide gels (10%–15%) and migrated at 150 V for 75 min in a minigel apparatus (BioRad Laboratories, Hercules, CA, USA). Proteins were transferred to nitrocellulose membranes with a Trans-Blot semidry transfer cell (BioRad Laboratories). Incubation of the membranes was performed on a SNAP i.d. 2.0 system (Millipore, Etobicoke, ON, Canada). Nonspecific sites were blocked for 20-min incubation in Odyssey blocking buffer (Li-CoR, Lincoln, NB, USA) [diluted 1:1 with phosphate-buffered saline (PBS)]. After PBS washing, membranes were incubated for 10 min with primary antibody 1:1000 phospho-Akt (S473) (Rabbit Ab Cell Signaling, Whitby, ON, Canada), phospho-Akt (T308) (Rabbit Ab Cell Signaling) and total Akt (Rabbit Ab Cell Signaling). After washing, the membranes were incubated for 10 min with secondary antibody 1:15,000 (anti-rabbit IRDye 800CW, Li-Cor). After washing, they were scanned with Odyssey Li-Cor Clx, and band intensities were analyzed with image studio (Li-CoR, Version 3.1). The same membranes were placed in stripping buffer (0.1 mol/L glycine and 1% sodium dodecyl sulfate, pH 2.0, for 1 h at room temperature) and reused with the same technique for phosphor-Akt/total Akt ratio determination.

2.5.3. Myeloperoxidase activity assay: neutrophil activity

Myeloperoxidase was assayed to assess neutrophil activity in the heart as described previously by Krawisz et al. [20]. Cardiac tissues were first weighed and added in hexadecytrimethylammonium bromide (HTAB) buffer (0.5% HTAB and 50 mM potassium phosphate, pH 6.0), then pulverized by sonication. The lysates went through three freeze/thaw cycles and were centrifuged at 10,000*g* for 15 min. To measure activity, 0.1 ml of supernatant was added to 2.9 ml of 50 mM sodium phosphate, pH 6.0, with 0.167 mg/ml of *o*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. Absorbance of the orange product was then measured every 10 s at 460 nm with a spectrophotometer for 2 min. The difference between maximum and minimum divided by 2 was generated to quantify neutrophil activity.