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Metabolites derived from omega-3 polyunsaturated fatty acids are important for cardioprotection

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ABSTRACT

Although controversial, some data suggest that omega-3 polyunsaturated fatty acids (PUFA) are beneficial to cardiovascular diseases, and could reduce infarct size. In parallel, we have reported that the administration of Resolvin D1 (RvD1), a metabolite of docosahexaenoic acid, an omega-3 PUFA, can reduce infarct size. The present study was designed to determine if the inhibition of two important enzymes involved in the formation of RvD1 from omega-3 PUFA could reduce the cardioprotective effect of omega-3 PUFA.

Sprague–Dawley rats were fed with a diet rich in omega-3 PUFA during 10 days before myocardial infarction (MI). Two days before MI, rats received a daily dose of Meloxicam, an inhibitor of cyclooxygenase-2, PD146176, an inhibitor of 15-lipoxygenase, both inhibitors or vehicle. MI was induced by the occlusion of the left coronary artery for 40 min followed by reperfusion. Infarct size and neutrophil accumulation were evaluated after 24 h of reperfusion while caspase-3, -8 and Akt activities were assessed at 30 min of reperfusion.

Rats receiving inhibitors, alone or in combination, showed a larger infarct size than those receiving omega-3 PUFA alone. Caspase-3 and -8 activities are higher in ischemic areas with inhibitors while Akt activity is diminished in groups treated with inhibitors. Moreover, the study showed that RvD1 restores cardioprotection when added to the inhibitors.

Results from this study indicate that the inhibition of the metabolism of Omega-3 PUFA attenuate their cardioprotective properties. Then, resolvins seem to be an important mediator in the cardioprotection conferred by omega-3 PUFA in our experimental model of MI.

1. Introduction

The controversy around the beneficial effect of the omega-3 polyunsaturated fatty acids (PUFA) in cardiovascular disease is still present. While some studies indicate that the consumption of omega-3 PUFA supplement has no significant effect on cardiac health (Rizos et al., 2012), other reveals a beneficial on infarct size (Abdukeyum et al., 2008; Nageswari et al., 1999). We have previously observed that a high omega-3 PUFA diet can result in a significant reduction of infarct size as compared to a high omega-6 PUFA diet (Rondeau et al., 2011).

Interestingly, it has been shown that the metabolism of long-chain omega-3 PUFA can generate molecules with anti-inflammatory actions (Serhan, 2010). Omega-3 PUFA eicosapentaenoic acid (EPA) and omega-3 PUFA docosahexaenoic acid (DHA) lead to resolvins (RvD) and protectins (Serhan and Chiang, 2008; Serhan et al., 2011). For instance, DHA could be converted to RvD by acetylated cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) but also by a COX-independent pathway through the 15-LOX (Serhan et al., 2002', 2004a', 2004b).

We have previously observed that the administration of RvD1, either before ischemia or a few min before the onset of reperfusion, significantly reduces infarct size (Gilbert et al., In press; Tran Quang et al., 2014). We have observed that a PI3K/Protein kinase B(Akt) inhibitor abrogates cardioprotection, indicating the importance of this pathway in the beneficial effect (Gilbert et al., In press).

Since myocardial infarction induces an inflammatory response (de Lorgeril et al., 1990; Simpson et al., 1988) associated with an increase of Cox-2 activity (Abbate et al., 2004) as well as the activation of other pathways (Song et al., 2014) involved in the synthesis of RvD1, we

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hypothesized that some of the beneficial effects of a high diet omega-3 PUFA may be related to the synthesis of RvD1. To test our hypothesis, we design this study to determine if in the presence of a high omega-3 PUFA diet, the inhibition of the Cox-2 and 15-LOX attenuates the beneficial effect observed by this diet in our experimental model of myocardial infarction.

2. Materials and methods

2.1. Experimental design

One hundred and nineteen 3-month-old Sprague–Dawley rats (Charles River Canada, St-Constant, QC, Canada), weighing 300–350 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 (21–22 °C temperature and 40–50% humidity), including a 12 h dark–light cycle beginning at 8:00 am. Chow pellets and tap water were available *ad libitum* throughout the study. An acclimatization period of 3 days after delivery by the supplier was imposed before the rats were randomly distributed into 1 of the 3 protocols.

2.1.1. Protocol 1

All rats were placed on a commercial standard diet rich in omega-3 PUFA (TD.08484 Harlan Teklad, Madison WI) for 10 days before MI induction. They were randomly assigned to one of the four groups: 1 – Vehicle (0.5 ml of vehicle (NaCl 0.9%)), 2 – an inhibitor of COX-2, Meloxicam, (1 mg/kg; AK Scientific, Inc (Union City, CA, USA)), 3 – an inhibitor of 15-LOX, PD146176, (5 mg/kg; Enzo Life Science (Farmingdale, NY, USA)) or both inhibitors Meloxicam (1 mg/kg) and PD146176 (5 mg/kg) dissolved in 0.5 ml NaCl 0.9%. Vehicle, Meloxicam and/or PD146176 were injected on the 8th and 9th day of the diet and 5 min before the MI induction. Concentrations of the inhibitors were chosen according to previous studies (Peskar et al., 2009; Tolba et al., 2014) and from our pilot study to confirm the inhibition of these enzymes with this regimen.

The animals were killed 24 h post-MI to measure infarct size and neutrophil activity. A subgroup of rats was killed 30 min after the onset of reperfusion for biochemical analysis (caspase-3, caspase-8, Akt and nuclear factor-kappaB (NF-κB) activation).

2.1.2. Protocol 2

All rats were placed on a commerical standard diet (Charles River 5075-U.S.), low in omega-3 PUFA, for 10 days before MI induction. They were randomly assigned to one of the four groups to receive 0.5 ml vehicle (NaCl 0.9%), Meloxicam (1 mg/kg), PD146176 (5 mg/kg) or Meloxicam (1 mg/kg) and PD146176 (5 mg/kg) dissolved in 0.5 ml NaCl 0.9%. Vehicle, Meloxicam and/or PD146176 were injected on the 8th and 9th day of the diet. On day 10 of the diet, 5 min before ischemia, Vehicle, Meloxicam and/or PD146176 were injected and all rats were sustained to a 40 min ischemia. The animals were killed 24 h post-MI to measure infarct size.

2.1.3. Protocol 3

A commerical diet rich in omega-3 PUFA (TD.08484 Harlan Teklad, Madison WI), the same as protocol 1, was given to the rats for 10 days before MI induction. They were randomly assigned into one of the 4 groups: group 1 – 0.5 ml vehicle (NaCl 0.9%), group 2 - Resolvin D1 0.1 μg (17(S)-RvD1 from Cayman Chemical (Ann Habor, MI, USA)), group 3 – Meloxicam (1 mg/kg) and PD146176 (5 mg/kg); group 4 – Meloxicam (1 mg/kg), PD146176 (5 mg/kg) and RvD1 (0.1 μg) dissolved in 0.5 ml NaCl 0.9%. Vehicle, Meloxicam and PD146176 were injected on the 8th and 9th day of the diet. On the 10th day of the diet, Vehicle, Meloxicam, PD146176 and RvD1 were injected 5 min before ischemia. The animals were killed 24 h post-MI to measure infarct size and RvD1 concentration (3–7 rats by group).

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 were monitored throughout the procedure. Left thoracotomy at the 5th 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 killed after 30 min of reperfusion or, with 24 h reperfusion, the thorax was closed with 2-0, 3-0 and 4-0 silk sutures (Syneture; Covidien) and the animals were given a subcutaneous antibiotic injection of 15,000 IU penicillin G (Duplocillin LA, Intervet Canada Ltd., Whitby, ON, Canada) as well as subcutaneous analgesic injection (2 mg/kg buprenorphine) before being returned to their respective cages.

2.3. Measurement of infarct size

After the reperfusion period (30 min or 24 h), the rats were restrained in a cone bag and rapidly decapitated. Their hearts were removed immediately and placed in a dish kept on crushed ice. In animals with 24 h reperfusion, the hearts were removed and washed with saline by retrograde perfusion *via* 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 4 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 the area of necrosis (I) from the AR. The different regions were carefully drawn on a glass plate, photocopied and cut. After that, the complete infarct region, area at risk and left ventricle (LV), were weighed separately to express MI as percentage of necrosis (I) of the AR ((I/AR) × 100) and AR as percentage of the LV area ((AR/LV) × 100). In animals with 30 min reperfusion, the ischemic region was divided into endocardial and epicardial areas. Samples were kept at −80 °C until needed for biochemical analysis.

2.4. Biochemical analysis

2.4.1. Caspase-3 and 8 activities

Caspase-3 and -8 activities were measured according to the protocol described previously (Rondeau et al., 2011). Tissues were homogenized by sonification 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 μg of protein (attested by the Bradford method) and fluorescent substrate (Ac-DEVD-AMC for caspase-3 and Ac-IETD-AMC for caspase-8) (40 μM). 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 an excitation wavelength of 365 nm for both caspase-3 and -8 and an emission wavelength of 465 and 430 for caspase-3 and caspase-8 activity respectively.