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Comparison of the effects of EPA and DHA alone or in combination in a murine model of myocardial infarction ☆

Ness Madingou [a,b], Kim Gilbert [a,b], Leandro Tomaro [a], Charles Prud'homme Touchette [a], François Trudeau [a], Samuel Fortin [c], Guy Rousseau [a,b,*]

[a] Centre de biomédecine, Hôpital du Sacré-Cœur de Montréal, 5400 boul. Gouin Ouest, Montréal, Québec, Canada H4J 1C5
[b] Département de pharmacologie, Université de Montréal, C.P. 6128 Succursale Centre-ville, Montréal, Québec, Canada H3C 3J7
[c] SCF Pharma, 235 Route du Fleuve Ouest, Ste-Luce, Québec, Canada G0K 1P0

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ABSTRACT

The aim of this project was to investigate the impact of two dietary omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), alone or in combination, on infarct size.

Adult, male Sprague-Dawley rats were fed for 14 days with different omega-3 diets. The animals were subjected to ischemia for 40 min followed by reperfusion. Infarct size, Akt (protein kinase B) activation level, caspase-3 activity and mitochondrial permeability transition pore (mPTP) opening were measured.

The results indicate that EPA or DHA alone significantly reduced infarct size compared to the other diets. Akt activity was increased in the group fed EPA or DHA alone, whereas no significant activation was observed in the other groups compared to no omega-3 PUFA. DHA alone reduced caspase-3 activity and conferred resistance to mPTP opening.

In conclusion, our results demonstrate that EPA and DHA are individually effective in diminishing infarct size in our experimental model while their combination is not.

Contents

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* Corresponding author at: Centre de biomédecine, Hôpital du Sacré-Coeur de Montréal, 5400 boul. Gouin Ouest, Montréal, Québec, Canada H4J 1C5.
E-mail address: guy.rousseau@umontreal.ca (G. Rousseau).

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1. Introduction

Cardiovascular diseases are major causes of mortality in industrialized countries [1]. Since many years, numerous approaches in addition to the reestablishment of the blood flow in the ischemic regions have been taken to curb the damage induced by ischemia. Experimentally, it has been noted that the activation of different signalling pathways, at the onset of reperfusion, is protective of the myocardium and results in smaller infarct size [2]. The reperfusion injury salvage kinase (RISK) pathway has been identified as one of them [2]. This pathway involves a series of protein kinases, such as Akt (protein kinase B), which converge on mitochondrial permeability transition pore (mPTP) opening [3–6]. In previous work, we saw that Akt is activated by omega-3 polyunsaturated fatty acids (PUFAs) and one of their metabolites, Resolvin D1 (RvD1), indicating linkage between the RISK pathway and the cardio-protection offered by them [7,8].

Two of the well-known omega-3 PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Both are usually present in our diet or in supplements. However, the individual effects of both are hypothetical since few side-by-side studies have been performed to compare them in the same model. Modulation of dietary omega-3 content is usually the result of increased EPA and DHA in different proportions, from which it is difficult to draw a conclusion on individual effects.

The present study was designed to determine if dietary DHA, EPA or both could influence infarct size evoked by coronary artery occlusion.

2. Methods

A total of 115 male 3-month-old Sprague-Dawley rats, weighing 300–350 g at the beginning of the experiments, were purchased from Charles River Canada (St-Constant, QC, Canada). They were handled in compliance with regulations of the local Animal Care Committee and the *Guidelines of the Canadian Council on Animal Care*. The animals were housed individually under constant conditions (21–22 °C temperature and 40–50% humidity), including a 12-h dark-light cycle beginning at 8 a.m. Chow pellets and tap water were available *ad libitum* throughout the study. The rats were randomly assigned to 1 of 5 groups: no-omega-3 PUFA, EPA 5 g/kg, DHA 5 g/kg, EPA+DHA 2.5 g/kg of each and EPA+DHA 5 g/kg of each. After 14 days on these diets, all groups underwent 40 min of left anterior descending coronary artery occlusion. Some animals were sacrificed after 24 h of reperfusion (n=8–9/group) to measure infarct size while others were euthanized 30 min after the onset of reperfusion (n=9/group) for biochemical analysis (caspase-3 and Akt activities) or at 15 min after the onset of reperfusion to measure mitochondrial permeability transition pore (mPTP) opening.

2.1. Diets

The diets were custom prepared in SCF Pharma's laboratory. They were prepared by thoroughly mixing 1400 g of powdered rat feed (LabDiet, Certified Rodent Diet 5002) with emulsion of appropriate weight of specific monoglyceride-omega-3 PUFA in 750 mL of water. Antioxidants were added during the preparation. The resulting wet diet was rolled and cut into pieces of approximately 1 cm^3. The cut pieces were dehydrated in a convection oven (350 °F, 25 min) to yield 1500 g of final diet.

2.2. In vivo surgical procedure

Anesthesia was induced by intraperitoneal ketamine/xylazine injection (60 and 10 mg/kg, respectively). The rats were subsequently intubated, and anesthesia was maintained under isoflurane (1–2%) ventilation. Electrocardiograms and heart rate (HR) were monitored throughout the procedure. Left thoracotomy at the 5th intercostal space allowed left anterior descending coronary artery occlusion with 4–0 silk suture (Syneture; Covidien, Mansfield, MA, USA) and plastic snare. Ischemia was confirmed by ST segment alterations, and the presence of ventricular sub-epicardial cyanosis. The suture was removed after 40 min of ischemia, permitting myocardial tissue reperfusion. The rats were sacrificed after 15 min, 30 min or 24 h of 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 15,000 IU penicillin G (Duplocillin LA, Intervet Canada Ltd., Whitby, ON, Canada) was injected subcutaneously for antibiotic prophylaxis with 2 mg/kg buprenorphine for analgesia, before being returned to their respective cages.

2.3. Hemodynamics data

HR was measured at different points during the experiment. Mean arterial pressure (MAP) was quantified by the tail cuff technique (Kent Scientific Corporation, Torrington, CT, USA) before occlusion, at 20 min of ischemia, at the onset of reperfusion and at 10 min of reperfusion. Pressure rate product (PRP) was calculated by the multiplication of HR and MAP/100.

2.4. Measurement of infarct size and tissue dissection

At the end of the reperfusion period, the rats were restrained in a cone bag and rapidly decapitated. The hearts of animals with 24-h reperfusion were removed immediately, 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 myocardial infarct (MI) induction (see above) to delimit 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 necrosis from the AR. The different regions were