Clinical Nutrition xxx (2016) 1–7

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Effect of parenteral infusion of fish oil-based lipid emulsion on systemic inflammatory cytokines and lung eicosanoid levels in experimental acute pancreatitis

Priscila Garla [a, *], Ricardo Garib [a], Raquel S. Torrinhas [a], Marcel C.C. Machado [a], Philip C. Calder [b], Dan L. Waitzberg [a]

[a] University of Sao Paulo, Faculty of Medicine (FMUSP), Department of Gastroenterology, Digestive Surgery Division – LIM 35 and University of Sao Paulo – NAPAN – Food and Nutrition Research Center, São Paulo, Brazil
[b] Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton and National Institute for Health Research Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom

ARTICLE INFO

Article history:
Received 4 May 2015
Accepted 10 December 2015

Keywords:
Omega-3 fatty acids
Fish oil
Lipid emulsion
Systemic inflammatory response
Eicosanoids
Experimental acute pancreatitis

SUMMARY

Parenteral fish oil lipid emulsion (FOLE) might mitigate inflammation after injury. Acute pancreatitis (AP) can occur following major surgery and is characterized by tissue and systemic release of inflammatory mediators that contributes to the systemic inflammatory response syndrome and multiple organ failure.
Aim: We evaluated the effect of short-term FOLE infusion before experimental induction of AP on systemic cytokine and lung eicosanoid profiles.
Methods: Lewis rats (n = 72) received parenteral infusion of FOLE (FO group) or saline (SS group), or remained without parenteral infusion (CG group) for 48 h. Thereafter, AP was induced by retrograde injection of sodium taurocholate into the pancreatic duct. Animals were sacrificed after 2, 12 and 24 h. Blood and lung samples were collected to assess serum inflammatory cytokines (Luminex) and tissue eicosanoids (ELISA), respectively.
Results: Serum TNF-α increased over time and serum IL-10 decreased from 12 to 24 h in CG group. In SS group serum TNF-α increased from 12 to 24 h ($p = 0.039$) and serum IL-10 decreased over time. Both CG and SS groups exhibited increased IL-6/IL-10 ratio ($p = 0.040$). From 12 to 24 h animals from FO group showed decreased serum IL-1 ($p < 0.001$), IL-4 ($p < 0.002$) and IL-6 ($p = 0.050$), and a trend towards increased IL-10 ($p = 0.060$). All experimental groups showed a trend towards increased PGE_2 and decreased LTB_4 in the lung at 24 compared with 12 h
Conclusion: Parenteral infusion of FOLE for 48 h before the induction of experimental AP appears to favorably influence the cytokine response without affecting lung eicosanoids at the time points measured. The use of FOLE to prevent and treat AP following major surgery needs to be further explored.

1. Introduction

Up to 10% of patients who receive traumatic insults, including complex types of gastrointestinal surgery or long periods of pancreatic ischemia during extensive cardiovascular surgery and cardiopulmonary bypass, may develop acute pancreatitis (AP) [1–3]. Procedures involving the handling and infusion of contrast agents in the common bile or pancreatic ducts, such as retrograde cholangiopancreatography, may also cause AP with an incidence ranging from 5 to 40% of patients [4–6]. Once initiated, AP contributes to increased morbidity and mortality, and its severity is related to the degree of injury and the severity of the associated systemic inflammatory response [7].

The inflammatory response plays a key role in development of AP and in the systemic complications of the disease (such as pancreatitis-associated lung injury), which are a main cause of mortality [7]. Clinical and experimental studies suggest that inflammatory cytokines are essential mediators of the pathophysiology of AP. Activation of polymorphonuclear granulocytes and

* Corresponding author. Laboratório de Investigação Médica – LIM 35, Faculdade de Medicina da USP, Av. Dr. Arnaldo, 455; Cerqueira César – CEP: 01246903, Sao Paulo, Brazil. Tel.: +55 11 30617459.
E-mail address: prigarla@gmail.com (P. Garla).

http://dx.doi.org/10.1016/j.clnu.2015.12.006
0261-5614/

monocytes, associated with release of the pro-inflammatory cytokines interleukin (IL)-1, IL-6 and tumor necrosis factor alpha (TNF-α) in the pancreatic parenchyma, is an early event during the development of AP and has been shown to contribute to the severity of the disease [7].

Nutrients with anti-inflammatory effects, including n-3 polyunsaturated fatty acids (PUFAs), have been studied in AP with the aim of minimizing the inflammation and improving clinical outcome [8–12]. Fish oil is a source of the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA competes with the n-6 PUFA arachidonic acid (AA) in the cyclooxygenase 2 (COX-2) pathway to produce odd-series eicosanoids with a lower pro-inflammatory potential than those of even-series derived from AA [13,14]. In turn, a change in eicosanoid production can influence other aspects of the inflammatory response including leukocyte infiltration and cytokine production [14]. Furthermore, n-3 PUFAs can reduce inflammatory cytokine production through non-eicosanoid related effects on the pathways that lead to activation of inflammatory cytokine production [14]. Finally, EPA and DHA give rise to mediators that are now recognized to play a central role in resolution of inflammation [15].

The anti-inflammatory effects of n-3 PUFAs rely upon their incorporation into the membrane phospholipids of inflammatory cells [13–15]. Intravenous (i.e. parenteral) administration of fish oil lipid emulsion (FOLE), as source of n-3 PUFAs, results in faster incorporation of these fatty acids into cell membranes compared to when they are administrated enterally [16]. Thus it is possible that the parenteral route may be advantageous in achieving modulation of inflammation by n-3 PUFAs in a short time. Therefore, the present study evaluated the impact of parenteral infusion of FOLE before the induction of experimental AP in rats on systemic and lung inflammatory mediator levels. We hypothesised that animals receiving FOLE would show a lower burden of inflammatory mediators.

2. Methods

2.1. Animals

Seventy-two adult male Lewis isogenic rats (300–350 g) were purchased from the Multidisciplinary Center for Research in Biological Science Laboratory Animal Area (Campinas, Sao Paulo, Brazil). Prior to the experimental procedures, the animals were adapted for 5 days in metabolic cages at a controlled room temperature (22 ± 2 °C) with a 12-h light–dark cycle and with free access to standard rodent chow (Nutrilav, Quimtia, Jundiaí, Brazil) and water. All experimental procedures were approved by the Research Ethical Committee, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil.

2.2. Intravenous access

Animals were anesthetized by the intraperitoneal injection of ketamine (100 mg/kg of body weight; Parke-Davis, Ache, São Paulo, Brazil) and xylazine (8 mg/kg of body weight; Bayer, Leverkusen, Germany). Intravenous access was achieved by jugular central venous catheterization (CVC) that was performed according to a standard technique and by using a specific catheter that allows its connection to a gyratory swivel apparatus, which ensured free mobility for the animals [17]. One group of animals did not receive any intravenous infusion for 48 h (CG group). The delivery of a total daily volume of 6 ml was controlled across all groups with a volumetric infusion pump (Colleague® – Baxter, California, USA). For that purpose, the SS infusion bags were prepared by adding a total of 6 ml of 0.9% saline solution, while the FOLE infusion bags were prepared by adding 0.4 g/kg body weight of FOLE (1 mL – Omegaven® 10%) plus 5 ml of saline solution. All animals had free access to standard oral diet and water *ad libitum* during this period.

2.3. Experimental acute pancreatitis

After 48 h of intravenous access, all animals were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg of body weight) and xylazine (8 mg/kg of body weight). The pancreas was exteriorized through an abdominal incision and the pancreatic duct catheterized using polyethylene tubing PE-50 (Biotechnological). AP was then induced by retrograde injection of 0.1 ml 3% sodium taurocholate solution (Sigma Chemical), according to a standard technique [18]. Following AP induction, animals were sacrificed after 2, 12 and 24 h by cardiac puncture for blood collection, after being properly anesthetized. Lung samples were collected from the animals sacrificed after 12 and 24 h.

2.4. Serum cytokine measurements

Blood samples were centrifuged at 1000 × g at 4 °C for 10 min to isolate serum. Concentrations of IL-1, IL-2, IL-4, IL-6, IL-10, and TNF-α were assessed in 500 μl serum by immunoassay multiplex microspheres, using a commercial kit for rats (07–65 K RECYTMAG, Genesis Ltd., Missouri, USA). Plates were read in a Luminex analyzer (Luminex, MiraiBio, Alameda, CA), according to the manufacturer's instructions [19].

2.5. Eicosanoid measurements

Lungs were washed in PBS and frozen in liquid nitrogen before storage at −80 °C for later analysis. Lungs were homogenized in phosphate buffered saline (PBS, 100 mg tissue/mL) and the concentrations of leukotriene B_4 (LTB_4), prostaglandin E_2 (PGE_2), thromboxane B_2 (TB_2) and lipoxin A_4 (LXA_4) were determined by using commercially available ELISA kits, according to the manufacturer's protocols [20].

2.6. Fatty acid measurements

Lipids were extracted from plasma and homogenized lungs with chloroform: methanol (2:1) and phospholipids were isolated by thin layer chromatography using a mixture of hexane: ethyl ether: acetic acid (90: 30: 1), according to the method of Folch et al. [21]. Fatty acid methyl esters were prepared by incubation with 140 g/L of methanol and boron trifluoride at 80 °C for 60 min. Subsequent to the extraction process, the fatty acid methyl esters were dried and separated by gas chromatography (Shimadzu Model GC-2010) with flame ionization detection, and an Omegawax 250 (Supelco) column. The operating conditions of the column corresponded to an initial temperature of 180 °C (1 min) and then 270 °C (5 min), with a total run time of 36 min. Fatty acid methyl esters were identified and quantified by comparison with external standards.

2.7. Statistical analysis

Data were analyzed using SPSS 18.0 for Windows software (SPSS, Chicago, IL, USA). They were analyzed by Kruskal–Wallis test and multiple comparisons between the groups were carried out with the Behrens–Fisher test. Lipid mediator concentrations were analyzed with the statistical software R 3.1.0 (Core Team, 2014). The nime package was used to adjust the statistical models. In all cases a value for $P < 0.05$ was taken to indicate a significant difference.

Please cite this article in press as: Garla P, et al., Effect of parenteral infusion of fish oil-based lipid emulsion on systemic inflammatory cytokines and lung eicosanoid levels in experimental acute pancreatitis, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2015.12.006