Prostaglandins, Leukotrienes and Essential Fatty Acids 112 (2016) 37–43

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Decreased n-6/n-3 polyunsaturated fatty acid ratio reduces chronic reflux esophagitis in rats

Jing-Jing Wei [a,1], Du-Peng Tang [a,1], Jing-Jing Xie [a], Li-Yong Yang [b], Ze-Hao Zhuang [a,*]

[a] Department of Gastroenterology, the First Affiliated Hospital of Fujian Medical University, China
[b] Department of Endocrinology, the First Affiliated Hospital of Fujian Medical University, China

ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form
27 June 2016
Accepted 15 August 2016

Keywords:
Polyunsaturated fatty acid
Reflux esophagitis
Lipid peroxidation
Dietary fat
Inflammation

ABSTRACT

Aim: To investigate the effect of dietary ratio of n-6/n-3 PUFAs on chronic reflux esophagitis (RE) and lipid peroxidation.
Method: Rat RE model were established and then fed on a diet contained different n-6/n-3 PUFA ratios (1:1.5, 5:1, 10:1) or received pure n-6 PUFA diet for 14 days. Esophageal pathological changes were evaluated using macroscopic examination and hematoxyline-eosin staining. IL-1β, IL-8, and TNFα mRNA and protein levels of were determined using RT-PCR and Western blotting, respectively. Malondialdehyde (MDA) and superoxide dismutase (SOD) levels were determined using ELISA.
Results: The severity of esophagitis was lowest in the PUFA$^{1:1.5}$ group ($P<0.05$). IL-1β, IL-8, and TNFα mRNA and protein and MDA levels were significantly increased in model groups with the increasing n-6/n-3 PUFA ratios. SOD levels were significantly decreased in all RE PUFA groups ($P<0.05$).
Conclusion: Esophageal injury and lipid peroxidation appeared to be ameliorated by increased n-3 PUFAs intake.

1. Introduction

Gastro-esophageal reflux disease (GERD) is an increasingly prevalent condition that the estimated prevalence of GERD in Eastern Asia was 2.5–6.3% in 2005 [1]. Between 2005 and 2010, the prevalence was estimated to have risen to 6.8–18.3% [1–3]. The condition has been shown to be associated with increased consumption of high-fat diet [4].

Polyunsaturated fatty acids (PUFAs) are important components of dietary fat. n-6 and n-3 fatty acids are two main types of PUFAs that are characterized by double bonds in the fatty acid chain. In a typical modern diet, the n-6: n-3 PUFA ratio is estimated to be > 10:1, due in large part to the limited sources of n-3 PUFA. Several studies have shown that high n-6: n-3 PUFA ratio in diet may promote esophageal inflammation associated with reflux esophagitis (RE), a typical complication of GERD [5]. However, the effect of dietary fat on the incidence of GERD is not well understood, probably owing to the complexity of the dietary patterns and dietary fat content. It is also unknown whether increased amounts of n-3 PUFAs in diet contribute to the development and progression of chronic inflammation in the esophagus.

Oxidative stress is closely related to chronic inflammation [6–8]. Oxidative stress can be determined by measuring the levels of malondialdehyde (MDA), which is a lipid peroxide generated by the reaction between oxygen free radicals and PUFAs [9]. The capacity for scavenging free radical or the anti-oxidant capacity can be estimated by measuring the levels of enzyme superoxide dismutase (SOD). SOD catalyzes conversion of the superoxide anion radical into hydrogen peroxide (H_2O_2) which is broken down to H_2O. PUFAs are targets of reactive oxygen species (ROS). Increased amounts of n-3 PUFAs may exacerbate lipid peroxidation under oxidative stress, as its unsaturated double bonds are more susceptible to oxidation [10,11]. On the other hand, n-3 PUFAs are known to abate oxidative stress by upregulating the capacity of cellular antioxidant defense system [6]. The effect of increased dietary intake of n-3 PUFAs on oxidative stress and inflammation remains to be elucidated.

In the present study, we sought to assess the effects and underlying mechanism of the different dietary ratio of n-6/n-3 PUFAs on chronic RE in a pyloric nylon loop-induced chronic acid reflux esophagitis (PNL-CARE) model. We found that low n-6: n-3 PUFA ratio in diet may reduce reflux related esophageal mucosal insult by upregulating antioxidant activity, and down-regulating lipid peroxidation. Reduction in the dietary ratio of n-6/n-3 PUFAs may be a beneficial strategy for management of GERD.

* Correspondence to: No. 20, Chazhong road, Fuzhou, Fujian, 350005, China.
E-mail addresses: weijingjing0208@163.com (J.-J. Wei), tangdupeng1989@163.com (D.-P. Tang), 328230221@qq.com (J.-J. Xie), yly_lm@sina.com (L.-Y. Yang), zhuang203@yeah.net (Z.-H. Zhuang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plefa.2016.08.003
0952-3278/

2. Materials and methods

2.1. Rat reflux esophagitis (RE) model and treatment

Healthy male Sprague-Dawley rats (2 months old) were sourced from the SLAC laboratory Animal Co. Ltd. (Shanghai, China). A month (30 days) prior to the treatment, the rats were housed in well-ventilated separate cages, exposed to 12 h light/dark cycle, an ambient temperature of 22 ± 2 °C, 60 ± 10% relative humidity, and ad libitum access to food and water.

A PNL-CARE model was established as described for PCASL-CARE (pyloric clip and section ligation induced chronic acid reflux esophagitis) model with modifications [12]. In brief, a 1.5 cm midline incision was made in upper abdomen. The forestomach was ligated with a 0.2 mm non-absorbable suture. Partial ligation of the pylorus to a diameter of 4.2 mm was achieved with a pyloric nylon loop. Animals received 1 mL of 5% metronidazole and 0.5 mL of gentamicin through the open abdomen. All animals were routinely housed for 14 days after surgery. Body weight and daily behavior were recorded. 14 days after surgery, animals were sacrificed by cervical dislocation. The esophageal tissues were obtained for further processing and analysis.

A total of 50 rats were randomly divided into five equal groups. Ninety five percent of dietary intake of rats in all groups consisted of lipid-free formula diet AIN-93 G; the remaining 5% was supplemented by different n-6: n-3 PUFA ratios in three groups: 1) $PUFA^{1:\ 1.5}$ (n-6: n-3 PUFA ratio 1:1.5); 2) $PUFA^{5:\ 1}$ (5:1) and 3) $PUFA^{10:\ 1}$ (10:1), respectively. Groups 4 and 5 received pure n-6 PUFA. Group 5 was labeled as the sham group and animals subjected to a sham operation; rats in the other 4 groups underwent surgery for establishment of RE model.

The ingredients of diet are listed in Table 1. "Jinlongyu" sunflower oil (Jiali Food and Oil Industry Ltd, Sichuan, China [linoleic acid; 70% n-6 PUFAs; no n-3 PUFAs]) was used as the source of n-6 PUFAs. n-3 PUFAs was obtained from fish oil (juvenile type, mainly containing EPA and DHA, < 1% n-6 PUFAs) (Hongyangsheng Biotechnology Ltd., Shandong, China). Diets were sterilized using ^{60}Co irradiation and were provided by Slake Experimental Animals Ltd, packaged under vacuum, and stored at −20 °C.

All experimental procedures and animal care protocols were approved by the Committee on Ethical Use of Animals at the First Affiliated Hospital of Fujian Medical University (Permit number SCXK [Hu]: 2007-0005).

2.2. Examination of esophageal inflammation

Rat esophagus tissues were collected on day 14 and longitudinally opened. Esophageal inflammation was recorded based on visual observation and histo-morphological examination. To examine histo-morphological changes, esophagus samples were fixed in 10% buffered-formaldehyde solution and sectioned in 5 μm thick. Tissue sections were stained with hematoxyline-eosin. Two pathologists, who were blinded to the group information, evaluated the esophageal specimens for the degree of mucosal damage according to the Chinese Society of Digestive Endoscopy GERD Guidelines. Squamous hyperlasia, lengthening of rete peg, and inflammatory infiltration were considered indicative of mild lesion. Presence of mucosal erosion was considered as a moderate lesion, while presence of mucosal ulcers as severe lesion. Epithelium thickness were measured and inflammatory cells were examined.

2.3. Quantitative real-time PCR analysis

Total RNA was isolated from esophageal tissues grounded in liquid nitrogen using Trizol reagent (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer's protocol. cDNA was synthesized using the Superscript II First Strand Synthesis System (Life Technologies, Gaithersburg, MD, USA). cDNA was then amplified by polymerase chain reaction (PCR) using primers specific to IL-1β (sense: 5′-TGTGATGTTCCCATTAGAC-3′; antisense: 5′-AATACCACTTGTTGGCTTA-3′), IL-8 (sense: 5′-ATGACTTCCAAGCTGGCCG-3′; antisense: 5′-CTCAGCCCT CTTCAAAAACTT-3′), TNFα(sense:5′-CCACGCTCTTCTGTCTACTG-3′; antisense: 5′-GCTACGGGCTTGTCACTC-3′), and β-actin (sense: 5′-TTCCAGCC TTCCTTCCTG-3′; antisense:5′-GGCATAGAGGTCTTT ACGG-3′).

Table 1
Ingredients and composition of diets for animals (g/kg).

Ingredient/group	n-6/n-3 = 10:1	n-6/n-3 = 5:1	n-6/n-3 = 1:1.5	n-6
Casein	140.000	140.000	140.000	140.000
Cysteine	1.800	1.800	1.800	1.800
Corn starch	435.192	435.192	435.192	435.192
Malt	125.000	125.000	125.000	125.000
Sucrose	158.000	158.000	158.000	158.000
Cellulose	50.000	50.000	50.000	50.000
Sunflower oil	45.670	42.000	25.330	50.000
Fish oil	4.330	8.000	24.670	0.000
Vitamin C	0.008	0.008	0.008	0.008
Premix	40.000	40.000	40.000	40.000

The condition for the logarithmic phase of amplification for each set of primers were determined in preliminary experiments, and included an initial step at 95 °C for 5 min, followed by 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s for 40 cycles, and a final extension step at 72 °C for 5 min β-actin was used as an internal control and as a standard reference to calculate relative levels of target genes. 2% agarose gel electrophoresis was employed for size separation of the PCR products. The size(s) of PCR products was determined by a gel documentation system (Ultra-Violet Product Limited, CA, USA). The relative expression levels were determined by comparing the density to that of the controls.

2.4. Western blotting

Esophageal tissue sample (100 mg) was homogenized with 1.0 mL of cell lysis buffer (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM ß-glycerophosphate, 1 mM Na_3VO_4, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 1 μM PMSF). Protein concentration was measured using D_C Protein Assay kit (Bio-Rad, Hercules, CA, US). Equal amounts of proteins (30 μg) were loaded and loaded in a SDS–polyacrylamide gel by electrophoresis, and transferred on to a PVDF membrane (Amersham, Piscataway, NJ, US). The membrane was then incubated with a primary antibody (anti- IL-1β, IL-8, TNFα, or β-actin, Santa Cruz, CA) for 1 h at room temperature (RT). After washing with TBST buffer, the membrane was incubated with an appropriate secondary antibody. Signals were detected and visualized by ECL system (Amersham, USA). Protein levels were evaluated through densitometry (how intense the stain is).

2.5. ELISA for MDA and SOD in esophageal tissue

0.5 g esophageal tissue homogenates were centrifuged (3000 rpm) at 4 °C for 15 min. MDA and SOD levels in the supernatant were determined using the corresponding assay kits (SOD and MDA assay kit, Nanjing Jiancheng Bioengineering Institute, China), according to the manufacturer's instructions.

MDA levels (nmoL/mg prot)=(Sample OD value − blank OD value)/(standard OD value − standard blank OD value) × standard concentration [10 nmoL/mL] ÷ protein concentration [mg prot/mL].