Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 55 (2006) 1590–1598
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Effects of eicosapentaenoic acid on the early stage of type 2 diabetic nephropathy in KKA^y/Ta mice: involvement of anti-inflammation and antioxidative stress

Minfang Zhang[a,b], Shinji Hagiwara[a], Masukazu Matsumoto[a], Leyi Gu[b], Mitsuo Tanimoto[a], Shinji Nakamura[a], Shigeru Kaneko[a], Tomohito Gohda[a], Jiaqi Qian[b], Satoshi Horikoshi[a], Yasuhiko Tomino[a,*]

[a]*Division of Nephrology, Department of Internal Medicine, Juntendo University School of Medicine, Tokyo 113-8421, Japan*
[b]*Department of Nephrology, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China*

Received 17 March 2006; accepted 20 July 2006

Abstract

Eicosapentaenoic acid (EPA) has been reported to have beneficial effects on the progression of various renal diseases including diabetic nephropathy; however, the precise mechanisms are not completely understood. We examined the effects of EPA on the early stage of type 2 diabetic nephropathy in KKA^y/Ta mice and the possible role of inflammation, oxidative stress, and growth factor in this process. KKA^y/Ta mice were divided into 2 groups. The treatment group was injected with EPA ethyl ester at 1 g/kg per day intraperitoneally from 12 to 20 weeks of age and the control group was injected with saline. Renal morphologic examinations were performed after 8 weeks of treatment. Glomerular macrophage infiltration and expression of monocyte chemoattractant protein 1, malondialdehyde (MDA), nitrotyrosine, transforming growth factor β1 (TGF-β1), and type I collagen were evaluated. Eicosapentaenoic acid decreased the levels of urinary albumin, serum triglyceride and MDA, and improved glucose intolerance in KKA^y/Ta mice. Morphometric analysis showed that accumulation of extracellular matrix and the tubulointerstitial fibrosis area were significantly decreased after treatment. Immunohistochemistry revealed that glomerular macrophage infiltration and the expression of MDA and nitrotyrosine in KKA^y/Ta mice were increased and were inhibited by EPA treatment. Protein and gene expression levels of monocyte chemoattractant protein 1, TGF-β1, and type I collagen, which were evaluated by immunohistochemistry and real-time reverse transcriptase–polymerase chain reaction, were down-regulated in the EPA treatment group. In conclusion, EPA improves type 2 diabetic nephropathy in KKA^y/Ta mice. This beneficial effect might be mediated by attenuation of metabolic abnormalities and inhibition of renal inflammation, oxidative stress, and TGF-β expression.

1. Introduction

Diabetic nephropathy is the leading cause of end-stage renal disease and the most frequent cause of mortality in patients with diabetes [1]. The slow and continuous decline in renal function is characterized by progressive glomerulosclerosis, tubulointerstitial injury, and renal fibrosis [2]. It is essential to identify more methods of treatment that can arrest the disease progression. Several studies have shown that a diet rich in n-3 polyunsaturated fatty acids (PUFA), specifically eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) in fish oil, might influence the progression of renal disease. The effects of dietary PUFA supplementation on renal injury have been reported in the 5/6 renal ablation model [3] and experimental focal glomerulosclerosis model [4]. Moreover, Donadio et al [5] demonstrated that EPA retarded the progression in patients with immunoglobulin A nephropathy. It was also reported that EPA showed beneficial effects in non–insulin-dependent diabetic patients with nephropathy by reducing albuminuria [6].

The mechanism through which PUFA exert their protective effects is still unclear. It appears that the basis is related to their actions on renal inflammation and fibrosis [7]. Many findings from animal and human studies show that PUFA have an anti-inflammatory effect. Polyunsaturated fatty acids

* Corresponding author. Tel.: +81 3 5802 1064 or 1065; fax: +81 3 5802 1064 or 1065.
E-mail address: yasu@med.juntendo.ac.jp (Y. Tomino).

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.07.019

may have direct effects on cellular production of major cytokine inflammation mediators, on endothelial dysfunction, and on leukocyte chemotaxis. Thus, PUFA treatment has been proposed for various inflammatory diseases such as rheumatoid arthritis and Crohn disease [8]. Diabetic nephropathy is frequently associated with an inflammatory status. Dalla Vestra et al [9] demonstrated that acute-phase markers of inflammation are associated with the severity of renal pathologic changes in diabetic patients with nephropathy. Monocyte chemoattractant protein 1 (MCP-1), which is the strongest known chemokine, may play an important role in the inflammation of renal diseases through recruiting and activating monocytes/macrophages from the circulation to inflammatory sites [10]. The inflammatory status, if not reversed, could accelerate the production of several cellular mediators such as transforming growth factor β (TGF-β) and the subsequent cell activation/proliferation and extracellular matrix (ECM) accumulation [11]. Therefore, the effect of PUFA on the expression of MCP-1 and TGF-β in diabetic nephropathy is worthy to be investigated.

Oxidative stress is another important factor involved in the progression of diabetic nephropathy. However, because PUFA are susceptible to be auto-oxidized and form the lipid peroxidation, there remains a theoretical concern with respect to the potential for increasing the oxidative stress after treatment [12]. However, Mori et al [13] reported that both EPA and DHA reduce oxidative stress in treated hypertensive type 2 diabetic subjects. To date, the data in vivo are inconclusive.

The KKA^y/Ta mice produced by transfection of the yellow obese gene (Ay) into KK/Ta mice are obese diabetic mice showing hyperglycemia, hypertriglyceridemia, hyperinsulinemia, and microalbuminuria. The pathologic changes of KKA^y/Ta mice are consistent with those in the early stage of human diabetic nephropathy, which include glomerular basement membrane thickening and expansion of mesangial matrix. The urinary albumin-creatinine ratio (ACR) in diabetic KKA^y/Ta mice is 250 to 350 mg/g creatinine (Cr) at 8 weeks of age and increases to 550 to 600 mg/g Cr at 16 weeks of age. Therefore, KKA^y/Ta mice are considered as a suitable model for the early stage of type 2 diabetic nephropathy [14].

Based on these findings, we aimed to assess whether EPA treatment could suppress the MCP-1, TGF-β, type I collagen expression, and glomerular macrophage infiltration in diabetic KKA^y/Ta mice. At the same time, the effects of EPA on oxidative stress, measured as serum MDA level and renal MDA and nitrotyrosine expression, were also examined.

2. Materials and methods

2.1. Animals and experimental design

Male KKA^y/Ta mice (7 weeks of age) were purchased from CLEA Japan (Tokyo, Japan). The mice were individually housed in plastic cages with free access to food (rodent pellet diet NMF; 1456 kJ [348 kcal]/100 g, containing 5.5% crude fat) and water throughout the experimental period. All mice were maintained in the same room under conventional conditions with a regular 12-hour light/dark cycle and temperature controlled at 24°C ± 1°C. KKA^y/Ta mice were randomly divided into 2 groups of 8 mice each. Administration of EPA was started at 12 weeks of age, which is considered as the early stage of diabetic nephropathy. The first group (treatment group) was injected with EPA ethyl ester at 1 g/kg per day intraperitoneally for 8 weeks. The second group (control group) was injected with saline [15]. Purified EPA ethyl ester was kindly provided by Mochida Pharmaceutical (Tokyo Japan). It was stored at −20°C inside the capsules (containing EPA943 mg and α-tocopherol 2 mg per capsule) and was freshly prepared before injection. KKA^y/Ta mice were killed at 20 weeks of age. Kidneys were removed for light microscopy, immunohistochemical examination, and RNA extraction.

2.2. Phenotypic characterizations

The body weight, blood pressure, fasting blood glucose levels, and ACR were measured at 12, 16, and 20 weeks of age. Serum triglyceride, total cholesterol, glucose tolerance, immunoreactive insulin (IRI), and MDA levels were measured at 20 weeks of age.

Blood pressure was measured at 11:00 AM by a noninvasive tail cuff and pulse transducer system (Softron BP-98A, Tokyo, Japan) after the mice were externally prewarmed for 10 minutes at 38°C. At least 3 to 6 recordings were taken for each measurement. Standard deviations of less than 5.0 were defined for the blood pressure levels. Urinary albumin and creatinine from samples collected for 24 hours using metabolic cages (mouse metabolic cage, CLEA Japan) were measured by immunoassay (DCA 2000 system, Bayer Diagnostics, Elkhart, IN). Serum total cholesterol and triglyceride were determined enzymatically by an autoanalyzer (Fuji Dry-Chem 5500, Fujifilm, Tokyo, Japan). Glucose tolerance was estimated by the intraperitoneal glucose tolerance test (IPGTT). It was performed by injection of glucose (2 g/kg in 20% solution) in overnight-fasted mice. Blood was obtained from the retro-orbital sinus at 0 (fasting blood glucose level) and 120 minutes after intraperitoneal glucose injection for measurement of the blood glucose and IRI levels. Glucose levels were measured using Glucocard (Kyoto Daiichi Kagaku, Kyoto, Japan). Immunoreactive insulin levels were measured by enzyme-linked immunosorbent assay (insulin enzyme-linked immunosorbent assay kit, Morinaga & Co, Tokyo, Japan).

Serum MDA was determined colorimetrically by a commercial kit (lipid peroxidation assay kit, Calbiochem, San Diego, CA) according to the manufacturer's instructions. Briefly, 0.65 mL of reagent 1 (7.7 mmol/L *N*-methyl-2-phenylindole in 75% acetonitrile and 25% methanol) was added to 0.2 mL of serum. After vortexing for 3 to 4 seconds and adding 0.15 mL of 12 N hydrochloric acid, samples were mixed and closed with a tight stopper and incubated at