Involvement of Nuclear Factor Kappa B in High-Fat Diet-Related Pancreatic Fibrosis in Rats

Ming-Xian Yan*, Hong-Bo Ren†, Yi Kou†, Min Meng‡, and Yan-Qing Li§

*Department of Gastroenterology, Shandong Qianfoshan Hospital, Shandong University School of Medicine, Shandong, †Department of Gastroenterology, Qilu Hospital of Shandong University, Shandong, ‡Department of Gastroenterology, Beijing Liangxiang Hospital, Beijing, and §Department of Gastroenterology, Anyang Hospital, Henan, China

Background/Aims: High-fat diets contribute to pancreatic fibrogenesis, but the pathogenesis remains unclear. This study investigated the role of nuclear factor kappa B (NF-kB) in high-fat diet-induced pancreatic fibrosis in rats. Methods: Male Wistar rats were fed a high-fat diet or standard normal chow for 20 weeks. Pancreatic fibrosis was determined by Sirius red staining. Immunohistochemical staining, reverse transcription-polymerase chain reaction and Western blotting were used to identify NF-kB-associated genes or protein expressions. Results: Inflammation, fat deposition, pancreatic stellate cell activation and fibrosis were observed in the pancreas of the high-fat diet group. NF-kB subunit p65 (NF-kB/p65) expression was localized to the nucleus, and intercellular adhesion molecule 1 (ICAM-1) was over-expressed. Pancreatic gene expression levels of NF-kB/p65, ICAM-1 and tumor necrosis factor alpha were all elevated significantly in rats fed a high-fat diet compared with control rats. Conclusions: NF-kB is involved in high-fat diet-related pancreatic fibrosis. (Gut Liver 2012;6:381-387)

Key Words: High-fat diet; Pancreatic fibrosis; NF-kappa B; Intercellular adhesion molecule 1; Tumor necrosis factor-alpha

INTRODUCTION

Prolonged high-fat diets intake is found harmful to pancreas. According to previous studies, high-fat diets can induce pancreatic endocrine and exocrine abnormalities, increased inflammatory cytokines in pancreatic tissues, and pancreatic stellate cell (PSC) activation and fibrogenesis. High-fat diets incite oxidative stress in pancreas, which has been shown to be involved in PSCs activation and pancreatic fibrosis. Although elevated levels of platelet-derived growth factor type beta and transforming growth factor beta 1 (TGF-β1) have been found in the pancreas in an animal model after high-fat diet feeding, the regulatory mechanisms and signaling pathways involved in this oxidative damage process have not been elucidated and our knowledge remains limited.

Nuclear factor kappa B (NF-kB) is an oxidative stress-sensitive transcription factor which modulates a wide variety of genes, including pro-inflammatory cytokines and adhesion molecules such as tumor necrosis factor alpha (TNF-α) and intercellular adhesion molecule 1 (ICAM-1). Quiescent PSCs can be stimulated by cytokines, growth factors and reactive oxygen species (ROS) to subsequently synthesize and secrete increased amounts of extracellular matrix. Activated PSCs promote autocrine factors including ICAM-1, TNF-α, and TGF-β in turn.

In view of the above considerations, we hypothesize that NF-kB might be involved in the deleterious effects on the pancreas that are due to chronic high-fat diets. In this present study, we fed rats a high-fat diet singly for 20 weeks, observed histological alterations, investigated some molecules expression that related to the NF-kB signaling pathways in the pancreas, and discuss the underlying implications of our results.

MATERIALS AND METHODS

1. Animal models

This study had the approval of the Ethics Committee of Shandong University. Twenty-four male Wistar rats (weighing 167 to 188 g, obtained from Shandong University Laboratory Animal Center) were used in the experiment. They were maintained in accordance with the Laboratory Animal Care and Use Regula-
tions of Shandong University. The rats received a regular rat chow for 1 week to acclimatize to their new environment, and were then divided into two dietary groups based comparable body weight. Rats in the control group (n=10) received a regular chow; rats in the treatment group (n=12) were fed a high-fat diet (2% cholesterol, 10% lard, and 88% regular chow as for the control group). All rats were fed for 20 weeks from the beginning of the experiment. Animals were sacrificed after fasting overnight and anesthetized by intraperitoneal injection with pentobarbital sodium (50 mg per kg body weight), at which time pancreas tissues were obtained.

2. Hematoxylin and eosin (H&E) and Sirius red staining

Samples of pancreas were formalin-fixed, paraffin-embedded, and cut into 5 μm thick sections and stained with H&E for histological observations. Inflammation score and fat deposition was evaluated as follows: 0, 0%; 1, 0% to 25%; 2, 25% to 50%; 3, >50%.

For collagen detection, sections were deparaffinized and immersed for 25 minutes in saturated aqueous picric acid containing 0.5% Sirius red to stain collagen fibers, and exposed to Harris hematoxylin for 3 minutes to stain nuclei. Under these conditions, the collagen fibrils appear red and the non-fibrotic areas appear blue. The fibrotic area was measured by ImageJ analysis software version 1.39n (National Institutes of Health, Bethesda, MD, USA) (http://rsb.info.nih.gov/ij/), and was expressed as fibrotic index (fibrotic index=area of pancreatic fibrosis/total area of specimen×100%).

To evaluate histological changes, three pancreas sections were randomly selected from each rat, and five non-overlapping fields were captured in every section for observation.

3. Immunohistochemical staining

Sections of pancreas were incubated with primary mouse anti-rat IgG antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight, and then incubated with biotinylated goat anti-mouse secondary antibodies and HRP-conjugated streptavidin (Santa Cruz Biotechnology) at room temperature for 30 minutes. The sections were then incubated with primary mouse antibody for NF-κB/p65, the primary antibody was diluted with 0.3% triton in PBS (v/v), and both cytoplasm- and nuclear-stained cells were considered positive. For observation, three pancreas sections were randomly selected from each rat and five non-overlapping fields were captured in every section for further analyze. The average number of NF-κB/p65 or ICAM-1 positive stained cells (brown) per high power field was reported. Areas staining positive for α smooth muscle actin (α-SMA) were measured by ImageJ software, and expressed as percentage of total area.

4. Gene expression

Gene expression was determined by reverse transcription-polymerase chain reaction (RT-PCR). PCR was performed with reaction mixtures containing dNTP, sense and antisense primers and TaqDNA polymerase (Takara, Shiga, Japan). β-actin was used as an internal standard control. PCR primers and conditions are as follows: NF-κB/p65: sense primer: 5'-ATG-GACGATCTGTTCCTCC-3', antisense primer: 5'-GTCTTAGTG-GTATCTGTGCT-3', fragment size: 170 bp, PCR condition: 94°C, 45''; 60°C, 45''; 72°C, 45'', 35 cycles; ICAM-1: sense primer: 5'-AGCCTCAGGGCTAAGAGGAC-3', antisense primer: 5'-AGGGGTCCAGAGAGGCTA-3', fragment size: 496, PCR condition: 94°C, 45''; 58°C, 45''; 72°C, 45'', 35 cycles; TNF-α: sense primer: 5'-CTGAGCAAAACCCAAAG-3', antisense primer: 5'-CTGACGGTTGGTGTTGA -3', fragment size: 193 bp, PCR condition: 94°C, 45''; 50°C, 45''; 72°C, 45'', 35 cycles; β-actin: sense primer: 5'-AGATCTCTGACCGAGGTTG-3', antisense primer: 5'-CAGCAGCTGTGGTGCTACAGG-3' fragment size: 327 bp, PCR condition: 94°C, 45''; 58°C, 45''; 72°C, 45'', 35 cycles.

PCR products were separated by gel electrophoresis (1.5% agarose stained with ethidium bromide). Specific bands were visualized by enhanced chemiluminescence. The intensity of the bands was analyzed using ImageJ software and standardized to the β-actin signal.

5. Western blotting

Pancreatic tissues were cut into small pieces, washed with PBS (pH7.4), and then homogenized in ice-cold lysis buffer (10 mM Heps, 10 mM KCl, 1.5 mM MgCl2, 1 mM dithiothreitol (DTT), 1.5 mM phenylmethylsulfonyl fluoride (PMSF), 20 mM NaF, 200 μM Na3VO4, and protease inhibitor cocktail). After keeping the sample on ice for 20 minutes, NP-40 was added to a final concentration of 0.5%, and then samples were incubated on ice for another 20 minutes and centrifuged at 10,000g for 2 minutes at 4°C. The supernatants were used as cytosolic extracts for measurements of ICAM-1. The pellets were washed with PBS and re-suspended in nuclear extraction buffer (20 mM Heps, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 1 mM DTT, 1.5 mM PMSF, 20 mM NaF, 200 μM Na3VO4, and protease inhibitor cocktail) for 20 minutes on ice and centrifuged at 15,000g for 15 minutes at 4°C. The supernatants containing nuclear protein were collected for NF-κB/p65 detection.

An equal amount of protein (20 μg) was loaded into different lanes and was separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The primary antibodies (Santa Cruz Biotechnology) were NF-κB/p65 (1:150) and ICAM-1 (1:200). A peroxidase-conjugated secondary antibody (1:1,250; Santa Cruz Biotechnology) was used, and the membrane was visualized by enhanced chemiluminescence. The intensity of the bands was quantified using ImageJ software and standardized