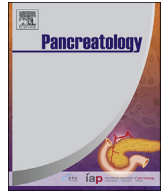Contents lists available at ScienceDirect

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The role of hypertriglyceridemia for acute kidney injury in the course of acute pancreatitis and an animal model

Congye Wu [a, b], Lei Zou [a], Shujing Shi [b], Zhihui Tong [a], Xiao Shen [a], Dongliang Yang [a], Lu Ke [a], Weiqin Li [a, *], Jieshou Li [a]

[a] Department of SICU, Research Institute of General Surgery, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China
[b] Department of Oncology, Nanjing First Hospital, Nanjing Medical University, Nanjing, China

A R T I C L E   I N F O

A B S T R A C T

Objective: The aim of this study was to investigate the role of hypertriglyceridemia for acute kidney injury (AKI) in the course of acute pancreatitis.
Methods: Patients with acute pancreatitis were retrospectively divided into four groups according to admission triglyceride: normal group, mild HTG group, moderate HTG group and severe HTG group. Clinical characteristics were compared among these groups. Wild type (WT) mice and Human ApoC III transgenic (ApoCIIItg) mice were used in the next animal experiments. Severe acute pancreatitis (SAP) model was established by retrograde injection of 0.5% sodium taurocholate (0.1 ml/100 g) from duodenum to pancreatic duct. Histological scores, serum amylase, creatinine, usea nitrogen were compared between WT mice and ApoCIIItg mice.
Results: Two hundred and sixty-two patients were classified into 4 groups: normal TG (104, 39.7%), mild HTG (72, 27.5%), moderate HTG (47, 17.9%), and severe HTG (39, 14.9%) groups. The proportions of AKI were 13.5% (14/104, normal), 13.9% (10/72, mild), 21.3% (10/47, moderate), and 38.5% (15/39, severe), respectively. After establishing SAP model, the levels of serum amylase ($P < 0.05$) and pancreatic histological score ($P < 0.05$) of ApoCIII-SAP-9h group were significantly higher than that of WT-SAP-9h group, respectively. ApoCIII-SAP-9h group had significantly higher levels of serum creatinine ($P < 0.001$), usea nitrogen ($P < 0.001$), and kidney histological score ($P < 0.05$) than that of WT-SAP-9h group, respectively.
Conclusions: Mild HTG has little adverse impact on disease severity of acute pancreatitis; severe HTG can aggravate kidney injury in the course of acute pancreatitis. ApoCIII-SAP mice have more serious pancreatic damage and kidney injury than WT-SAP mice.
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Introduction

Hypertriglyceridemia (HTG) is an accompanying condition in acute pancreatitis (AP) accounting from 2% to 26% [1]. It has been regarded as one of the most important issues, relating to the incidence, disease course and recurrence of AP [2,3]. Although lots of studies focus on HTG as an etiology of AP, the relationship of HTG and disease severity is not well established.

A recent systemic review indicated studies comparing the severity of hypertriglyceridemic AP with disease caused by other etiologies are heterogeneous and scarce [1]. In the study by Balachandra et al. [4], HTG had no significant correlation with the clinical severity of AP. Fortson et al. [5] reported that the prevalence of local complications in patients with HTG was similar to AP from other causes. However, Goyal et al. [6] found hypertriglyceridemic AP had greater severity of disease and less favorable outcomes than alcoholic pancreatitis. Elevated serum TG is independently and proportionally correlated with persistent organ failure regardless of etiology of AP [7]. Several other studies also showed that patients with HTG tended to have more severe disease course of AP [8–12]. An important reason of the heterogenicity is that few studies classify the level of serum TG more particular.

Acute kidney injury (AKI) takes a proportion of 20–25% of all AP population, and the total mortality of AP patients with AKI is about 25% [13,14]. Our previous study indicated HTG is an independent

risk factor for AKI in the early phase of AP [15]. We would further classify HTG into more detailed groups, and investigate the relationship of HTG and AKI. Havel et al. [3] supposed that free fatty acids (FFAs) hydrolyzed by pancreatic lipase from triglyceride (TG) is a potential cause of hypertriglyceridemic pancreatitis. Kimura et al. [16] also found pancreatic damage initiated by different pathogenetic pathways can be worsen by TG in animal models. We proposed that a similar pathophysiology may occur in hypertriglyceridemic pancreatitis related AKI.

The experimental studies almost unanimously confirm that HTG can aggravate AP in animal models. High-fat diet feeding is the most common method to simulate HTG condition. However, the serum level of TG can be only slightly higher than normal range in the above condition, and accordingly, it does not much conform to the clinical characteristics of hypertriglyceridemic AP. Human ApoC III transgenic (ApoCIIItg) mice were well adopted in the study for hypertriglyceridemic AP as an ideal animal model of primary severe HTG [17]. We would investigate the role of HTG for AP-related AKI from clinical study and animal experiments.

Materials and methods

Clinical study

Patients

From July 2009 to July 2014, patients with a diagnosis of AP admitted to the department of general surgery of Jinling Hospital were retrospectively included and analyzed. AP was diagnosed according to the serum amylase (Amy) level, combined with clinical presentations and image findings from computed tomography. The severity of AP were classified according to the revised Atlanta criteria [18]. Inclusion criteria were AP patients admitted to our hospital within 48 h of disease onset. Patients with recurrent AP and those lacking necessary clinical and biochemical data were excluded.

Data collection and clinical manifestations

Demographic parameters included age, gender, body mass index (BMI), history of smoking and alcohol abuse. Chronic comorbidities included hypertension, diabetes, HTG, biliary diseases, cardiovascular diseases, respiratory diseases and chronic renal diseases. Demographic parameters and chronic comorbidities were collected and analyzed. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Data were recorded on standard admission proformas and supplemented by retrospective chart review from the hospital laboratory database. Organ functions were evaluated on three organ systems and organ failure was defined as acute respiratory distress syndrome (ARDS) ($PaO_2/FiO_2 \leq 300$ mmHg), AKI (creatinine ≥ 2.0 mg/dl) and shock (need for vasoactive agent). The incidence of infected pancreatic necrosis, requirement for surgical intervention, length of intensive care unit (ICU) and hospital stay as well as mortality of the AP patients were also recorded.

Gradation of serum TG

Patients were divided into four groups according to admission serum TG: normal (<150 mg/dL), mild HTG (150–500 mg/dL), moderate HTG (500–1000 mg/dL), and severe HTG (>1000 mg/dL) groups.

Animal experiments

Animals and groups

Eight-week-old male wild type (WT) ICR mice and male ApoC-IIItg mice in ICR background were obtained from Institute of Cardiovascular Sciences, School of Basic Medical Sciences, Peking University Health Science Center. In ApoCIIItg group, only mice with serum TG more than 500 mg/dL were kept for next experiments. All mice were maintained in a temperature-controlled room (22 ± 1 °C) with a 12 h light/dark cycle for adaptation of 2 weeks. Both 30 WT mice and 30 ApoCIIItg mice were randomly divided into 5 groups: Control group, SAP-3h group, SAP-6h group, Sham-9h group, and SAP-9h group. Each group had 6 mice. All the animals were deprived of food and drinking water for 3 h before operation.

Model of SAP

Mice in control group were sacrificed under normal condition. SAP model was established by retrograde injection of 0.5% sodium taurocholate (0.1 ml/100 g weight) from duodenum to pancreatic duct as method of Perides et al. [19]. Sham group was established using normal saline by the same way. All mice underwent surgery under 2% pentobarbital sodium (0.1 ml/100 g) intraperitoneal injection anesthesia and sterile conditions.

Tissue samples

Pancreas and kidneys were quickly collected into liquid nitrogen, and stored at −80 °C for analysis. A tail of the head of the pancreas and the extracted kidney were fixed in 10% buffered formalin, and embedded in paraffin for morphological evaluation by H&E staining. Sections were slided to 5 μm-thick pieces and coded.

Pancreas morphological evaluation

The degree of pancreatic injury by light microscopy was based on severity of edema, inflammatory cell infiltration, and cell death. A pathologist blinded to the experiment evaluated the pathologic scores according to Schmidt's criteria [20].

Kidney morphological evaluation

The coded kidney specimens were stained with hematoxylin and eosin and examined in blinded fashion. Histological changes were evaluated by quantitative measurement of tubulointerstitial injury, which was assessed by counting the number of necrotic and apoptotic cells, loss of tubular brush border, tubular dilatation, cast formation, and neutrophil infiltration. The scoring was 0 = none; 1 = 0–10%; 2 = 11–25%; 3 = 26–45%; 4 = 46–75%; and 5 = 76–100% [21].

Assessment of blood chemistries

Blood was sampled from mouse eyes when mice were sacrificed, and was centrifuged at 15,000 rpm for 5 min in a microfuge. Total plasma TG was measured by the L type TG H kit (Jiancheng, Nanjing). High concentration of serum TG could interfere the evaluation of other biomarkers. Density-gradient ultracentrifugation was used to separate plasma lipoprotein for further detecting. The plasma samples were centrifuged for 18 h at 5 °C at 40,000 rpm in a Ti-50 fixed-angle rotor (Beckman Instruments, Geneva, Switzerland). After ultracentrifugation, serum Amy, creatinine (Scr) and blood urea nitrogen (BUN) were detected to evaluated pancreas damage and renal functions. Serum Amy level was determined using a colorimetric assay kit (Jiancheng, Nanjing). Scr and BUN concentrations were determined by the Scr and BUN Companion kit (Exocell).

Statistical analysis

Statistical analysis was performed using SPSS version 12 statistical software. Continuous variables were expressed as mean ± SEM or as medians with interquartile rang, and are