Hyperlipidemia intensifies cerulein-induced acute pancreatitis associated with activation of protein kinase C in rats

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AIM: To investigate the effects of hyperlipidemia on acute pancreatitis (AP) and the possible mechanisms.

METHODS: Rat models of hyperlipidemia and AP were established by Triton WR1339 and cerulein respectively. Human albumin was used to treat AP complicated by hyperlipidemia. In each group, we compared the histological score, volume of ascites, ratio of pancreatic wet/dry weight, serum amylase (AMY) and pancreatic acinar cell apoptosis. The level of protein kinase C (PKC) membrane translocation in pancreatic tissue was detected by Western blot.

RESULTS: In the hyperlipidemia model established by Triton WR1339, triglyceride (TG) increased remarkably and reached its peak 6 h after injection, and most rats developed mild acute pancreatitis. Histological score, volume of ascites, ratio of wet/dry weight and serum AMY in AP animals with hyperlipidemia were obviously higher than those in AP animals (P < 0.05) and decreased after albumin therapy but not significantly (P > 0.05). Apoptotic cells detected by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) increased in AP animals with hyperlipidemia and did not change distinctly after albumin therapy. PKC membrane translocation level increased in AP animals with hyperlipidemia and decreased remarkably after albumin therapy (P < 0.05).

CONCLUSION: Hyperlipidemia may induce AP or intensify pancreatic injury. Albumin therapy can not alleviate pancreatic lesion effectively. PKC activation may be one mechanism by which AP is intensified by hyperlipidemia.
bile/pancreatic duct respectively and found that the isolate pancreatic damage is increased by TG perfusion. However, some reports suggest that hyperlipidemia could not aggravate the course of AP. Paye et al[10] prepared an AP model with cerulein and injected TG simultaneously, and found that histological alterations, pancreatic enzyme level and ascites in these models do not differ from those in AP patients without TG injection. To approve this result further, the authors collected very low density lipoprotein (VLDL) from AP patients and found that the VLDL intravenous infusion for hypertriglyceridemia does not alter the course of AP[11]. Therefore the role of hyperlipidemia in acute pancreatitis seems questionable.

The mechanism by which hyperlipidemia leads to AP is still unclear. A well-accepted mechanism proposed by Havel et al[12] is that unbound FFAs are toxic and could produce acinar cells or capillary injury. Increased concentration of chylomicrons in pancreatic capillaries causes capillary plugging and leads to ischemia and acidosis. FFAs can induce activation of trypsinogen and initiate AP. Recently, it was reported that FFAs are toxic to pancreatic β-cells leading to diabetes and insulin resistance syndrome. The toxicity is associated with abnormalities in the insulin signaling cascade and may be mediated by FFA activation of protein kinase C (PKC)[13]. Also, there is abundant PKC in pancreatic acinar cells[14]. Other similar mechanisms of FFAs may damage acinar cells.

The aim of the present work was to determine whether endogenous hyperlipidemia aggravates in vivo cerulein-induced edematous AP and activates PKC in pancreas of rats.

MATERIALS AND METHODS

Animals
Male Sprague-Dawley rats, weighing 250-270 g were used. Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The rats were maintained at 23 °C in a 12 h light dark cycle, with free access to water and standard rat chow. The rats were fasted for 12 h prior to the experiment, but with free access to water. They were anesthetized with chloral hydrate (300 mg/kg intraperitoneal injection). Body temperature was monitored and maintained at 37.5 ± 1 °C using a heating device.

Models of hyperlipidemia, AP, and AP with hyperlipidemia
Hyperlipidemia was induced by intravenous injection of 10% Triton WR1339 solution (TyoloxAPol, Sigma, USA) through the left external iliac vein. Triton WR1339, a non-ionic surface active detergent leads to an increase of endogenous TG and total cholesterol (TCH) blood concentrations[15, 16]. We determined the required amounts of Triton WR1339 with two doses of 100 mg/kg and 400 mg/kg body weight respectively. A volume of 400 mg/kg Triton WR1339 solution could induce maximal hyperlipidemia. The non-hyperlipidemic animals received an injection of physiological saline solution intravenously instead of Triton WR1339.

AP was induced by 4 h abdominal subcutaneous injection of cerulein (Sigma, American, 20 µg/kg in 0.5 mL of saline vehicle) as previously described[17].

To explore the influence of hyperlipidemia on acute experimental pancreatitis, we used five different groups of rats with 12 rats in each group. The animals of the first group [C00] received 1.0 mL saline (sal) solution, and the second group [C00] received 1.0 mL of 10% Triton WR1339 (tri) intravenously. In these two groups [C00 and C00] no pancreatitis was induced. AP was induced in the third group (AP) and the fourth group (HAP) as described above. The animals of group HAP received an injection of 1.0 mL of Triton WR1339 intravenously 6 h before pancreatitis was induced with cerulein infusion. Since albumin could effectively combine FFAs in circulation to prevent cellular damage[18], in the fifth treatment group (THAP), the rats received an injection of 1.25 g/kg albumin intravenously after pancreatitis was induced.

Analysis of serum lipid, AMY activity and histology
Blood samples were taken from all animals before and 3, 6, 9 h after induction of hyperlipidemia or AP with hyperlipidemia. The samples were used to measure TG, TCH, AMY activity. Three and six hours after induction of AP, the rats were sacrificed by aortic puncture and blood samples taken for plasma biochemical measurements. A complete macroscopic examination of the peritoneal cavity and ascites was immediately performed, and the pancreas was removed using a standardized monobloc surgical technique. The pancreas was divided into three parts. The pancreatic head was fixed in Bouin’s solution, embedded in paraffin, sliced, and stained with hematoxylin and eosin. The pancreas body was put into liquid nitrogen for PKC analysis. To better estimate the amount of edema, the remaining pancreatic tissue was weighed and dessicated for 24 h at 173 °C to measure its dry weight. We used the wet to dry ratio to quantify edema formation.

To quantify the histological changes, two pathologists judged the tissue samples using a point score as previously described[19, 20]. Plasma AMY concentrations were determined after plasma dilution (1/20) by the colorimetric assay AMYL (Boehringer Mannheim, Germany). Plasma TG was measured using the colorimetric assay TG GPOPAP (Boehringer). Detection of apoptosis
Recent studies indicate that mild acute pancreatitis is related to the extent of acinar cell apoptosis[21-23]. A pancreatic tissue section was prepared and assayed with the in situ cell death detection kit, POD (Boehringer Mannheim, Germany) following the manufacturer’s instructions. Slides were counterstained with hematoxylin and viewed under a light microscope. Activation of protein kinase C
PKC activation was determined in both cytosolic and particulate fractions as previously described[24]. Frozen pancreatic tissue (50 mg) was rapidly thawed and homogenized at 4 °C in lysis buffer A (mM: Tris-HCl 50; pH 7.4; NaCl 150; EDTA 2; EGTA 2; DTT 2; Na py-
rophosphate 5; KF 50; PMSF 1; 1 mg/mL: Aprotinin, Leupeptin, Pepstatin A). Homogenates were centrifuged at 100 000 r/min for 30 min at 4 ℃ in an Optima TLX ultracentrifuge to yield cytosol. The pellet fractions were resuspended in 100 mL of buffer A containing 0.5% Nonidet P-40, sonicated and centrifuged again. The resulting supernatants taken as the particulate fractions with the cytosolic fractions were assayed for PKC immunoreactivities.

Throughout the study, protein concentrations were determined by the Lowry's method with bovine serum albumin as standard. Aliquots of the cell lysates containing 100 g of total protein were resolved in 10% SDS-PAGE and transferred to PVDF membranes (Amersham Biosciences, Inc.). The membranes were blocked in TBS-Triton-X 100 (0.2% v/v, pH 7.4) containing 5% milk for 2 h at room temperature followed by incubation with PKC antibody at the dilution described by manufacturer's instructions at 4 ℃ overnight. The membranes were incubated with anti-goat horseradish peroxidase complex (diluted 1:2000) for 2 h at room temperature. The proteins on the membranes were exposed to X-ray films at multiple time points and visualized by ECL detection system (Amersham Biosciences, Inc).

**Statistical analysis**

Data were expressed as mean ± SD and analyzed using Student's t test or Student's Newman Keuls-test by SPSS 11.0 software. Quantitative analysis for immunoblot was done by scanning the x-ray film and determined using the Fotodyne Gel-Pro analyzer program.

**RESULTS**

**Changes of serum lipid, AMY and pathology of pancreas in Ctr group**

The animals receiving 100 mg/kg and 400 mg/kg Triton WR1339 had high TG and TCH. The serum TG increased most obviously and reached its peak 6 h after Triton WR1339 infusion with induction of chylomicronemia (Figure 1). The TG value increased 10-fold and 20-fold after 100 mg/kg and 400 mg/kg Triton WR 1339 infusion respectively, whereas the serum TCH increased only 2-fold and 3-fold (Figure 2). The serum AMY activities did not significantly increase after induction of hyperlipidemia (P > 0.05). The animals did not demonstrate ascites and pancreatic edema in macroscopy. Nine rats (9/12) showed mild pancreatic edema, whereas no histological changes occurred in the saline-treated animals (group C_sal).

**Effects of hyperlipidemia on pancreatic histology in AP rats**

The pancreatic changes included interstitial edema, increased interlobular interstice and infiltration of some inflammatory cells in the animals 3 h after cerulein infusion. The pancreas of AP animals 6 h after cerulein injection had serious edema with tensioned capsules and separated small lobuli. Wide lobular and acinar interstices, anamorphic acinar structure, intracytoplasmic vacuolization and

![Figure 1](https://example.com/figure1.png)

*Figure 1* Development of chylomicronemia after Triton WR1339 injection.

![Figure 2](https://example.com/figure2.png)

*Figure 2* Curves of serum TG and TCH induced by Triton WR 1339 in rats.

![Figure 3](https://example.com/figure3.png)

*Figure 3* Microscopic pancreatic changes in rats with AP induced by cerulein (A) and hyperlipidemic AP induced by cerulein and Triton WR1339 (B) 6 h after injection (HE × 200).
infiltrated inflammatory cells were observed (Figure 3). After 3 h, the pancreatic damage was intensified in the animals of group HAP, and some apoptotic bodies (inclusion) and more inflammatory cells appeared. There were scattered erythrocytes and inflammatory cells, distended small veins in the pancreas 6 h after HAP model was induced (Figure 3). There were no pathological changes after albumin therapy.

Pancreatic histological score, volume of ascites and pancreatic wet/dry ratio in rats

The animals receiving cerulein (group Ctri) had significantly (P < 0.05) higher pancreatic histological score, volume of ascites and pancreatic wet/dry ratio than the control group. The pancreatic histological score, volume of ascites and pancreatic wet/dry ratio were significantly higher (P < 0.05) in the AP animals with hyperlipidemia (group HAP) than in the animals receiving only cerulein infusion (group Ctri). After albumin treatment (group THAP) these parameters decreased slightly but were higher in the 6 h group than in the 3 h group (P > 0.05). These results suggested that hyperlipidemia could aggravate pancreatic edema of the AP animals and albumin therapy was not obviously effective (Table 1).

Serum TG, TCH and AMY in rats

The serum TG and TCH concentration increased 1-2 folds in the cerulein-induced AP animals. There were no statistical differences between group AP and group Ctri (P > 0.05). The hyperlipidemic AP animals had significantly higher serum TG and TCH values than the rats receiving Triton WR1339 (group Ctri) injection. The serum TG and TCH concentration in the animals with albumin therapy (group THAP) decreased slightly. The AMY activities in the hyperlipidemic group Ctri remained unchanged throughout the investigation, and increased obviously (P < 0.05) in the AP group. In comparison with the AP group, the AMY values of group HAP and THAP were significantly higher (P < 0.05). This result demonstrated that AP could induce mild hyperlipidemia. Albumin could not decrease the circulating lipid and AMY concentration (Table 2).

Apoptosis of pancreatic cell in rats

It was reported that the extent of acinar cell apoptosis is related to the severity of mild AP[22]. Careful biochemical and morphological examination of experimental models of AP has shown that severe AP is associated with necrosis, whereas mild acute pancreatitis is associated with apoptotic cell death[20,21]. In our study, the TUNEL methods was used for in situ apoptotic acinar cell identification. The apoptotic cells were not visible in the pancreas of control group, but a few apoptotic cells (brown) were visible in cerulein-induced AP animals. A subpopulation of apoptotic cells scattered throughout the tissue section was stained brown Figure 4, but the apoptotic cells did not markedly decrease after albumin therapy.

Translocation of PKC in pancreatic tissue

To confirm the activation of PKC, we examined the translocation of the enzyme from the cytosol to the particulate membrane by Western blot. The cytosolic PKC gradually decreased in the control group Ctri, hyperlipidemic group Ctri, cerulein-induced AP group and HAP group. After albumin treatment the cytosolic PKC increased remarkably in group THAP. However, the particulate PKC gradually increased in the above four groups and decreased after albumin therapy (Figure 5). The degree of PKC translocation was evaluated by the proportion of particulate PKC in the total PKC as previously described[23]. The translocation rate of PKC was significantly higher (P < 0.05) in hyper-

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Table 1 Effects of cerulein and albumin therapy on histological pancreatic lesions, volume of ascites and ratio of pancreatic wet/dry weight (mean ± SD)

| Group | Time | Histological score | Ascites(ml) | Wet/dry |
|-------|------|--------------------|-------------|--------|
| C(sal) | 3 h  | 0.33 ± 0.52        | 0           | 1.35 ± 0.09 |
|       | 6 h  | 0.33 ± 0.52        | 0           | 1.35 ± 0.09 |
| AP    | 3 h  | 3.50 ± 1.38        | 0.87 ± 0.58 | 1.67 ± 0.05 |
|       | 6 h  | 5.33 ± 1.03        | 1.58 ± 0.92 | 1.76 ± 0.14 |
| HAP   | 3 h  | 5.17 ± 1.17        | 2.08 ± 0.58 | 1.95 ± 0.44 |
|       | 6 h  | 6.83 ± 1.16        | 4.12 ± 1.58 | 2.15 ± 0.39 |
| THAP  | 3 h  | 4.83 ± 1.16        | 1.87 ± 0.93 | 1.72 ± 0.14 |
|       | 6 h  | 6.67 ± 1.21        | 3.68 ± 1.42 | 1.88 ± 0.33 |

*aP < 0.05 vs control group, †P < 0.05 vs cerulein group.

Table 2 Effects of albumin therapy on serum TG, TCH and AMY in rats (mean ± SD)

| Group   | Time | TG          | TCH          | AMY          |
|---------|------|-------------|--------------|--------------|
| C(sal)  | -    | 47.00 ± 23.38 | 26.25 ± 2.36 | 1658.50 ± 335.21 |
| AP      | 3 h  | 63.66 ± 11.52 | 61.00 ± 6.00 | 5128.66 ± 259.65 |
|         | 6 h  | 72.66 ± 22.65 | 67.00 ± 8.71 | 6641.00 ± 213.75 |
| C(tri)  | 3 h  | 899.51 ± 240.52 | 76.03 ± 16.14 | 1652.55 ± 300.46 |
|         | 6 h  | 797.07 ± 356.18 | 60.51 ± 33.49 | 1253.72 ± 254.80 |
| HAP     | 3 h  | 1604.16 ± 248.10† | 264.33 ± 50.33† | 18390.00 ± 7158.77† |
|         | 6 h  | 1506.66 ± 327.72† | 230.33 ± 31.87† | 1161.66 ± 453.67† |
| THAP    | 3 h  | 1396.83 ± 658.65 | 221.00 ± 60.46 | 20473.33 ± 12005.46 |
|         | 6 h  | 1167.33 ± 228.28 | 211.00 ± 14.02 | 16524.39 ± 5273.52 |

*aP < 0.05, †P < 0.05 vs tripton group.

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Figure 4 Detection of apoptotic cells by TUNEL in rats with AP induced by cerulein (A) and hyperlipidemic AP induced by cerulean and Triton WR1339 (B) counterstained with hematoxylin (H × 200).

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![Image](https://example.com/image.png)
Effects of Triton hyperlipidemia, caerulein pancreatitis, AP with

Accordingly, it is very important to decrease circulating lipid in clinical patients with AP. In our studies, the pancreatic damage aggravated by hyperlipidemia was not prevented by simultaneous perfusion of albumin, which might be due to the fact that the concentration of albumin in the inflamed pancreas may be too low or there may be other mechanisms of pancreatic injury. It was reported that FFAs could inhibit division and proliferation of pancreatic endocrine cells by activating PKC and induce apoptosis of pancreatic β-cells. As a result insulin secretion is decreased. There is abundant PKC in pancreatic acinar cells. Our study showed that FFAs could cause injury of pancreatic exocrine cells. PKC activity of pancreas increased in the hyperlipidemia and AP animals. The PKC activity changes were consistent with the degree of pancreatic injury, suggesting that PKC may participate in the course of hyperlipidemia inducing or aggravating pancreatic injury of AP. The PKC activity in hyperlipidemic AP animals was decreased significantly (P < 0.01) after albumin therapy, which implies that albumin may reduce the PKC activation through binding to FFAs. Although PKC activation decreased obviously (P < 0.05) after albumin therapy, the pancreatic injury was slightly relieved (P > 0.05), suggesting that PKC activation may be an important aspect of hyperlipidemia aggravating AP, and there may be other mechanisms of hyperlipidemia-induced AP.

DISCUSSION

Hypertriglyceridemia is an etiological factor in patients with AP while TG level exceeding 1000 mg/dL or lactescent serum is a necessary condition for hyperlipidemic AP diagnosis. Triton WR1339 could increase serum TG and is an ideal drug to establish hyperlipidemic animal model with TG elevation.

The rats without pancreatitis showed no clinical differences after Triton WR1339 infusion compared with saline infusion. Macroscopically, the pancreas of the hyperlipidemic and normal animals showed no difference. Microscopically, the animals treated with Triton WR1339 showed smooth edema, whereas no changes occurred in the non-hyperlipidemic animals, suggesting that severe hyperlipidemia may cause mild AP. The serum AMY activities were also slightly increased after hyperlipidemia induction, but there was no significant difference (P > 0.05). To investigate the effect of hyperlipidemia on AP, we established the AP model when the endogenous hyperlipidemia induced by Triton WR1339 reached its maximal value. Theoretically, significant influence of hyperlipidemia on AP would appear. We produced the hyperlipidemic AP animal model by injecting cerulein 6 h after Triton WR1339 infusion. It was reported that albumin could effectively alleviate the toxicity of TG to pancreatic cells. A medium dose of albumin was injected through external iliac vein in the hyperlipidemia AP animals to observe the changes of pancreatic damage. As a result, hyperlipidemia aggravated pancreatic edema, volume of ascites and pancreatic cell apoptosis. The serum lipid in the hyperlipidemic AP animals increased compared with that in the hyperlipidemic animals, suggesting that hyperlipidemia may aggravate the course of AP while AP may exacerbate hyperlipidemia. There is a vicious circle between hyperlipidemia and AP. Accordingly, it is very important to decrease circulating

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S- Editor Guo SY  L- Editor Wang XL  E- Editor Bai SH