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Original article

Investigation for role of tissue factor and blood coagulation system in severe acute pancreatitis and associated liver injury

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ABSTRACT

This study aims to investigate the molecular mechanisms underlying the pathogenesis of severe acute pancreatitis (SAP) and SAP-associated liver injury, we performed an association analysis of the functions of tissue factor (TF) and blood coagulation system in both SAP patients and mouse SAP model. Our results showed that serum TF and tissue factor-microparticle (TF-MP) levels were highly up-regulated in both SAP patients and SAP mouse model, which was accompanied by the dysfunction of blood coagulation system. Besides, TF expression was also highly up-regulated in the Kupffer cells (KCs) of SAP mouse model. After inhibiting KCs in SAP mouse model, the amelioration of blood coagulation system functions was associated with the decrease in serum TF and TF-MPs levels, and the reduction of SAP-associated liver injury was associated with the decrease of TF expression in KCs. In conclusion, the dis-regulated TF expression and associated dysfunction of blood coagulation system are critical factors for the pathogenesis of SAP and SAP-associated liver injury. TF may serve as a potential and effective target for treating SAP and SAP-associated liver injury.

1. Introduction

Acute pancreatitis (AP) is a severe abdomen disease characterized by the activation of Trypsin in pancreas, which will cause pancreatic tissue self-digestion, edema, hemorrhage and even necrotic inflammation [1,2]. The patients with severe acute pancreatitis (SAP) suffer from severe pancreatic hemorrhage and necrosis which are usually accompanied by liver injury, infection, peritonitis and shock [3,4]. Therefore, the mortality of SAP is high.

The blood leaving pancreas is processed by the liver before returning to the heart, and the liver is frequently injured extrapancreatic organ in AP patients. As early as 1984, Blamey et al., report that 80% of the AP patients suffer from liver injury, and its severity is positively correlated with the progression of AP [5]. The pathological changes in liver cells can be observed in AP patients. Conversely, liver injury can contribute to the progression of AP [6,7]. Clinically, liver injury is an important indicator of AP severity and has significant predictive value for AP prognosis. Kupffer cells (KCs) account for 80–90% of the total cell number in the entire monocyte-macrophage system. They are the largest fixed macrophage population in human body. Previous studies indicate that KCs may contribute to AP-associated liver injury [8–10].

Currently, the pathogeneses of SAP and SAP-associated liver injury are not well-understood. Recent studies suggest that microcirculation has critical roles in AP occurrence and development [11,12]. The disturbance of pancreatic microcirculation is an important factor for the transformation from moderate acute pancreatitis (MAP) to SAP and the main cause of multiple organ failures and even death. Actively correcting or ameliorating the dysfunction of microcirculation can significantly reduce the severity of AP and improve AP prognosis. The imbalance between blood coagulation and fibrinolysis systems can largely contribute to the disturbance of pancreatic microcirculation. Abnormal blood

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coagulation system can be observed during early AP and is obvious at SAP [13,14]. Although both blood coagulation and fibrinolysis systems can be activated during AP, the functions of fibrinolysis system are relatively insufficient. The exact mechanism underlying the dysfunction of coagulation and fibrinolysis during AP remains unclear.

Tissue factor (TF) is a transmembrane protein mainly expressed in extravascular tissues [15,16]. Recent studies suggest that TF is involved in both extrinsic coagulation pathway and intrinsic coagulation pathway [16,17]. During microcirculation, the major vector of TF is TF-microparticles (TF-MPs) which are released from activated mononuclear cells, endothelial cells and platelets, etc. by exocytosis. After the stimulation by lipopolysaccharide (LPS), tumor necrosis factor α (TNF-α) or interleukin-1 (IL-1), etc., mononuclear cells can have high TF expression level. TF can then be transferred to platelet and participate in pathological blood coagulation process.

Therefore, to understand the pathogenesis of SAP and SAP-associated liver injury, we simultaneously analyzed serum TF/TF-MP levels, KC TF/TF-MP levels and the functional changes of blood coagulation system in both SAP patients and SAP mouse model. In addition, we also examined the impacts of KC inhibition on KC TF/TF-MP level and the associated changes of blood coagulation system and liver injury in SAP mouse model. This study may further reveal the relationship among TF level, blood coagulation system and the pathogenesis of SAP and SAP-associated liver injury. Besides, it may provide valuable clues on how to treat SAP and SAP-associated liver injury.

2. Materials and methods

2.1. Subjects

A total of 40 SAP patients in the Second Affiliated Hospital of Chongqing Medical University from May 2013 to May 2014 were enrolled in this study. There were 16 male cases, and 24 female cases. The mean age of the patients was 64.2 ± 6.8 years. In addition, 40 health old people (20 male cases and 20 female cases. The mean age was 63 ± 6.5 years) receiving physical examination in the same hospital during the same period were served as the control group. There was no significant difference in gender and age between SAP and control group. All procedures performed in the experiments involving human participants were in accordance with the ethical standards of Chongqing Medical University Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consents were obtained from all individual participants included in this study. AP diagnosis and classification were performed according to a new international classification system for acute pancreatitis severity [4]. SAP was diagnosed if patients had clinical and biochemical changes of AP and met at least one of the following conditions: local complications (pancreatic necrosis, pseudocyst or pancreatic abscess), organ failure, Ranson score ≥3, APACHEII score ≥8, Balthazar CT classification D or E.

Exclusion criteria: 1) occurred less than 72 h after admission; 2) with malignant tumor; 3) oral administration of the drugs influencing coagulation system, such as anticoagulant drugs; 4) children and pregnant women; 5) with hematological system

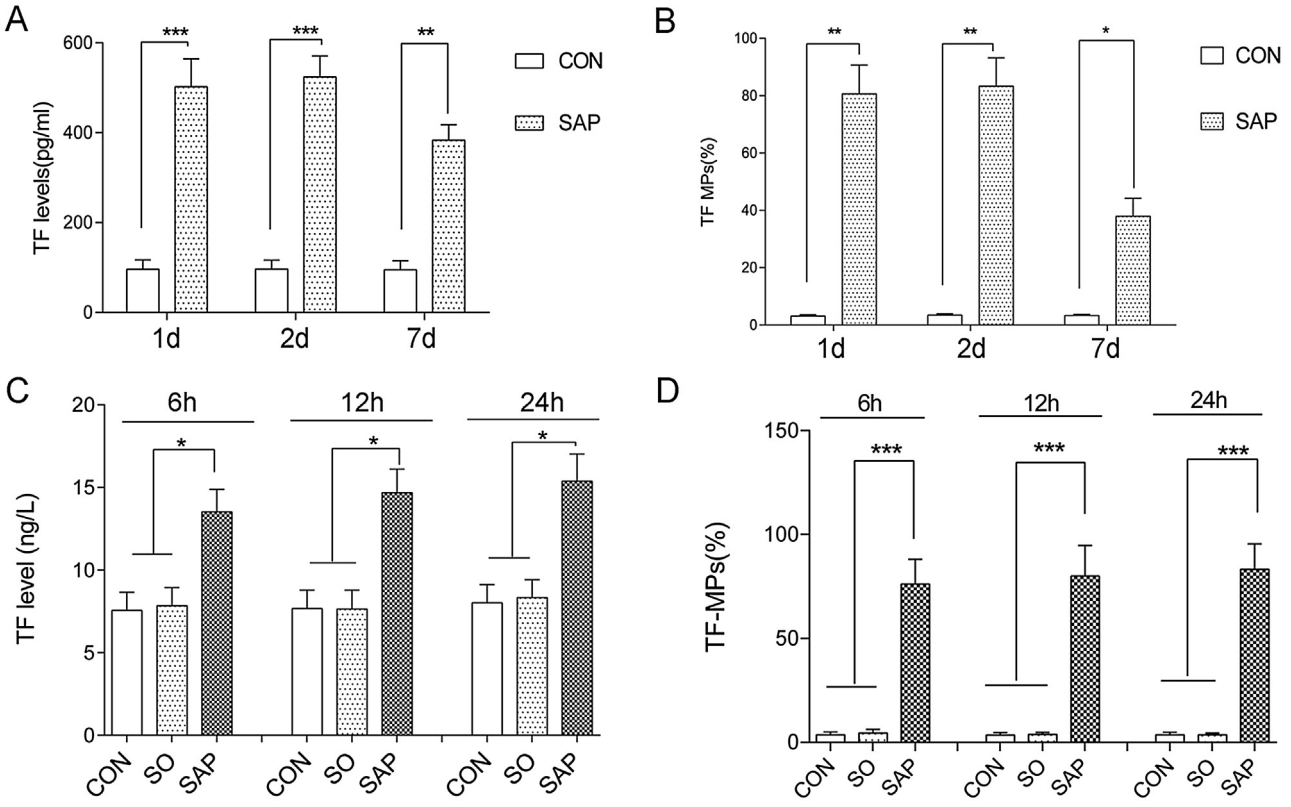

Fig. 1. Serum TF and TF-MP levels are highly up-regulated in both SAP patients and SAP mouse model. A ELISA showed that the TF levels in SAP patients were much higher than those in healthy control subjects at 1d, 2d and 7d after admission (3–5 folds, $p < 0.01$). B FCM showed that the TF-MP levels in SAP patients were also much higher than those in healthy control subjects at the above timepoints (10–25 folds, $p < 0.05$). At 6 h, 12 h and 24 h after the establishment of SAP mouse model, ELISA and FCM showed that TF (C) and TF-MP (D) levels were both significantly higher than those in normal control mice and SO mice ($p < 0.01$). n.s., no significant difference. *, $P < 0.05$. **, $P < 0.01$. ***, $P < 0.001$.