Original Article

Early changes of plasma angiopoietin-2 in patients with multiple trauma

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BACKGROUND: This study was undertaken to investigate the early changes of plasma levels of angiopoietin-2 (Ang-2) in patients with multiple trauma and the relations of plasma Ang-2, endothelial injury, and prognosis.

METHODS: This study comprised 59 patients with multiple trauma who had been treated at the emergency department of Liao Cheng People's Hospital from January 2008 to January 2010. Among them, 36 were male and 23 female. Their average age was 32.3 ± 11.5 years. The 59 patients were divided into a severe trauma group (ISS ≥ 16 points, 29 patients) and a slight trauma group (ISS < 16 points, 30 patients) by injury severity score (ISS). Thirty healthy people aged more than 18 years with an average of 33.5 ± 10.6 years served as controls (19 male and 11 female). Peripheral blood (10 mL) was collected within 10 minutes after the patients arrived at the emergency department, and plasma was separated from the blood. Enzyme-linked immunosorbent assay (ELISA) was applied to detect the levels of angiopoietin 2, thrombomodulin (TM), and Von willebrand factor (vWF).

RESULTS: The level of Ang-2 in the severe trauma group (ISS score ≥ 16 points) was significantly higher than that in the slight trauma group (ISS score < 16 points) ($P < 0.05$). The levels in the two groups were significantly higher than those in the control group ($P < 0.05$). The levels of angiopoietin-2 in deaths were significantly higher than those in survivors ($P < 0.05$). The levels of angiopoietin-2 were significantly correlated with the levels of vWF and TM ($P < 0.05$).

CONCLUSIONS: The plasma levels of Ang-2 are significantly higher after multiple trauma, and correlated with the degree of trauma severity. The levels of angiopoietin-2 are correlated with endothelial injury after multiple trauma, and are important values for the prognosis of patients with multiple trauma.

KEY WORDS: Multiple trauma; Angiopoietin-2; Endothelial injury; Prognosis

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INTRODUCTION

Angiopoietin-2 (Ang-2) is released by vascular endothelial cells and is an important cytokine that participates in physiological activities and pathophysiological events of endothelial cells. It has been shown that the plasma level of Ang-2 increases in patients with sepsis.^[1] But little is known about the role of Ang-2 early after trauma. The aim of the present study was to measure plasma levels of Ang-2 in trauma patients immediately after injury and to further understand the role of Ang-2 in trauma and its clinical significance.

METHODS

Patients

The patients with multiple trauma who had been treated at the emergency department of Liao Cheng People's Hospital from January 2008 to January 2010 were enrolled. Those patients who had been admitted to the department within 1 hour after trauma and at age of over 18 years were eligible for enrollment. Those with heart, brain, liver, kidney and other organic diseases, diabetes, hypertension, tumor, acute and chronic infectious diseases, severe brain injury (GCS < 12 points),

and use of anticoagulant and thrombolytic drugs two weeks before trauma were excluded.

Among the 59 patients, 36 were male and 23 female. Their average age was 32.3 ± 11.5 years. They were divided into a severe trauma group ($ISS \geq 16$ points, 29 patients) and a slight trauma group ($ISS < 16$ points, 30 patients) by injury severity score (ISS). The study protocol was approved by the Ethics Committee of the hospital and written informed consent was provided by relatives of the participants.

Thirty healthy volunteers (19 males and 11 females) served as controls. They were at age > 18 years with an average age of 33.5 ± 10.6 years.

Methods

Ten mL peripheral blood was taken from each trauma patient within 10 minutes after arrival at the emergency department. It was added with sodium citrate in tubes, mixed, centrifuged at 3000 r/min for 10 minutes. After separation of plasma, it was immediately placed in a refrigerator at -80°C for use. All measurements were made in a blinded way by an investigator.

Ang-2 was measured using enzyme-linked immunosorbent assay (ELISA). ELISA kit was purchased from R&D systems. The plasma level of thrombomodulin (TM) was measured using ELISA. ELISA kit was purchased from French DIA-CLONE Company. Both were measured using ALISEI an automatic ELISA analyzer produced by the Italian SEAC. Von willebrand factor (vWF) was measured by immune turbidimetry using STA an automatic coagulation analyzer produced by the French STAGO Company. STAGO Company's package kit was used in strict accordance with the instructions. Plasma levels of Ang-2, TM and Von willebrand factor in the control group were measured similarly.

Statistical analysis

SPSS13.0 software was used for statistical analysis, and data were expressed as mean $\pm$ standard deviation. Two-group analysis was performed with Student's *t* test. The differences between the groups were analyzed by ANOVA and Student-Newman-Keuls procedure. Correlation was assessed by Pearson's product-moment correlation coefficient. A *P* value ≤ 0.05 was chosen to represent statistical significance.

RESULTS

The plasma levels of Ang-2 in the severe trauma group ($ISS \geq 16$ points) were significantly higher

Table 1. Comparison of plasma levels of Ang-2, plasma vWF, and TM among the severe trauma group, slight trauma group and control group

Groups	<i>n</i>	Ang-2 (pg/mL)	vWF(%)	TM(ng/mL)
Severe trauma	29	$2900 \pm 210^{* \#}$	270 ± 65	58 ± 10
Slight trauma	30	$2100 \pm 190^{*}$	210 ± 60	49 ± 7
Control	30	1900 ± 200	120 ± 40	28 ± 5

Compared with the control group, $^{*}P < 0.05$; compared with the slight trauma group, $^{\#}P < 0.05$

than those in the slight trauma group ($ISS < 16$ points) ($P < 0.05$). The levels in the severe and slight trauma groups were higher than those in the control group ($P < 0.05$). The levels of Ang-2 were significantly correlated with the plasma levels of vWF and TM ($P < 0.05$) (Table 1). The plasma levels of Ang-2 in deaths were higher than those in survivors (3100 ± 230 vs. 2150 ± 195 , $P < 0.05$).

DISCUSSION

Trauma is one of the major causes leading to death. In China, the total number of deaths caused by all kinds of trauma each year has reached 70 million. It accounts for a quarter of the death population.^[2] It is estimated that the mortality caused by trauma will increase worldwide in recent years. Uncontrollable hemorrhage and severe injury of the central nervous system are responsible for the majority of trauma-related deaths in the first 24 hours. After this period, multi-organ failure instead of hemorrhage is considered as a major cause of trauma-related death.^[3] Endothelial damage has been shown to play a major role in the development of organ injuries, representing an independent parameter for poor clinical outcome in critically ill patients.^[4] The damage of the endothelium and extracellular matrix also plays a vital role in the pathogenesis of sepsis and multiple organ dysfunction syndrome (MODS).^[5] Impaired function of the vascular barrier may increase extravascular leakage of liquid, decrease systemic angiotasis and cause collapse of microcirculation, which lead to shock, acute respiratory distress syndrome (ARDS) and MODS.^[6]

In cases of severe trauma, vascular endothelial cells are vulnerable and can release vascular active substances and adhesion molecules, which participate in blood coagulation and inflammation. Ang-2 is an important cytokine secreted by vascular endothelial cells and participates in physiological activities and pathophysiological events of endothelial cells.^[7]

Ang-2 and angiopoietin-2 (Ang-1) are antagonistic ligands that bind with the Tie2 receptor, which is

almost exclusively expressed by endothelial cells. Ang-2 binds to the Tie-2 receptor but does not cause autophosphorylation of the Tie2 receptor while blocking the Ang-1/Tie-2 signaling and disrupting the endothelial barrier to enhance vascular leakage. Hence Ang-2 acts as a blood vessel destabilizing cytokine and disrupting the endothelial barrier.^[8] The plasma levels of Ang-2 were found to be elevated in patients with sepsis.^[1,9] Ang-2, increasing the vascular permeability, plays an important role in the pathogenesis of lung injury and pulmonary dysfunction, and in patients with acute lung injury and sepsis, high levels of circulating Ang-2 are associated with increased vascular leakage.^[1,9,10] The plasma levels of Ang-2 are positively related to vascular leakage and pulmonary dysfunction throughout the course of septic shock.^[11,12]

In the present study, the plasma levels of Ang-2 were significantly higher early after trauma and correlated with the degree of trauma severity. Ang-2 was released to blood early after trauma.

Our study demonstrates the plasma levels of Ang-2 are significantly correlated with the plasma levels of Von willebrand factor (vWF) and thrombomodulin (TM) after trauma. vWF and TM are endothelia-related markers and reflect endothelial cell injury from different aspects.^[13] TM is a cell surface glycoprotein and presents in vascular endothelial cells specifically. It is considered as a molecular marker of endothelial injury.^[13] vWF is a indispensable protein to make platelet adhere to the subendothelial matrix. The high levels of vWF may promote platelet adhesion, aggregation, and increase blood viscosity, thus leading to platelet thrombus formation. High vWF level is a sign of vascular endothelial cell injury. Pre-made vWF and Ang-2 proteins are typically stored in Weibel-Palade bodies and rapidly release upon stimulation of the endothelium. The exocytosis of Weibel-Palade bodies can be induced by multiple factors like hypoxia, thrombin and complement,^[14] which often occur after severe trauma.

The present study shows that the plasma levels of Ang-2 are significantly correlated with plasma levels of vWF and TM after trauma. This finding indicates that endothelial cells are activated and damaged early after trauma. Therefore Ang-2 could be a marker of endothelial activation and injury in trauma patients. Ang-2 may play an important role in the pathological process of increased vascular permeability induced by vascular endothelial activation and injury in trauma patients.

In the present study, the plasma levels of Ang-2 in deaths were significantly higher than in survivors. In

critically ill patients, higher Ang-2 levels were correlated with increased mortality.^[15] Studies^[1,11,15,16] have demonstrated that Ang-2 levels are elevated at the onset of critical illness in septic and non-septic patients with risk of ALI/ARDS, and that they are related to vascular permeability and pulmonary dysfunction. In trauma patients, trauma-induced MODS is the leading cause of death.^[17] Ang-2 is of significance in the pathogenesis and disease progression after trauma. It is either a marker of endothelial activation and damage or of predictive value for prognosis. Thus further studies are needed to elucidate the exact mechanism of Ang-2 in trauma and whether Tie-2 receptors are therapeutically effective.

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Ethical approval: The study was approved by the Ethics Committee of the hospital and written informed consent was provided by relatives of the participants.

Conflicts of interest: No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Contributors: Weng HB proposed the study and wrote the paper. All authors contributed to the design and interpretation of the study and to further drafts.

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