

Plasma levels of tumor necrotic factor- α and interleukin-6, -8 during orthotopic liver transplantation and their relations to postoperative pulmonary complications

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BACKGROUND: Pulmonary complications after orthotopic liver transplantation (OLT) include high morbidity and mortality. Experimental data have suggested hepatic ischemia and reperfusion are induced by pro-inflammatory cytokines. The high level of inflammatory cytokines might additionally influence pulmonary capillary fluid filtration. The objectives of this study were to measure the concentrations of tumor necrotic factor- α (TNF- α), interleukin-6 (IL-6) and interleukin-8 (IL-8) during OLT and to investigate the relationship between these cytokines and postoperative pulmonary complications.

METHODS: Twenty-two patients undergoing OLT were divided into two groups according to whether they had postoperative pulmonary complications: group A consisting of 8 patients with postoperative pulmonary complications, and group B consisting of 14 patients without postoperative pulmonary complications. Enzyme-linked immunoassay (ELISA) was used to determine serum TNF- α , IL-6 and IL-8. Blood samples were taken at the beginning of operation (T_0), clamping and cross-clamping of the inferior cava and portal vein (T_1 , T_2), 90 minutes and 3 hours after reperfusion (T_3 , T_4) and 24 hours after operation (T_5).

RESULTS: The level of PaO₂/FiO₂ in group A was lower than that in group B ($P < 0.05$). The concentrations of TNF- α , IL-6 and IL-8 in the two groups increased rapidly at T_2 , peaked at T_3 , decreased rapidly after T_3 until 24 hours after operation. The concentrations of TNF- α , IL-6 and IL-8 in group A were higher than those in group B at T_2 , T_3 , and T_4 ($P < 0.05$).

CONCLUSION: After un-clamping of the inferior cava and portal vein, the serum concentrations of TNF- α , IL-6 and IL-8 increased may be related to pulmonary injury after hepatic ischemic reperfusion.

(*Hepatobiliary Pancreat Dis Int* 2004; 3: 38-41)

KEY WORDS: liver transplantation; ischemic-reperfusion; tumor necrotic factor- α ; interleukin-6; interleukin-8; postoperative complication

Introduction

End-stage liver disease necessitating orthotopic liver transplantation (OLT) is frequently associated with an impaired efficacy of gas exchange.^[1-4] Major factors involved in abnormal oxygen uptake include intrapulmonary vasodilatation and shunting, ventilation-perfusion mismatch, pleural effusions and ascites. Although these changes are, for the most part, spontaneously reversible after transplantation,^[5-7] sporadic cases of respiratory failure do occur,^[8,9] requiring prolonged mechanical ventilation with negative effects on both treatment outcome and expenses.

In addition to a variety of causes such as anesthesia induced muscle paralysis, insufficient relief of postoperative pain, and accidental phrenic nerve injury,^[10] other pathophysiologic mechanisms of impaired lung function may exist.

Experimental studies have demonstrated that vasoactive mediators are swept into circulation from the splanchnic region and the implanted graft after reperfusion of OLT,^[11,12] some of them, eg. oxygen free radicals and cytokines, which contribute to tissue damage and endothelial disintegrity, have been identified.^[13-18] But there have been no related clinical reports on OLT.

The objectives of this study were to determine tumor necrotic factor- α (TNF- α), changes of interleukin-6 (IL-6) and interleukin-8 (IL-8) during OLT and to ascertain whether there are relations between

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changes of these cytokines and pulmonary complications after operation.

Methods

Twenty-two patients with end-stage liver diseases scheduled for elective OLT without veno-venous bypass were studied. Inclusion criteria for liver transplantation included absence of cardiac, renal or cerebrovascular diseases. Patients with a history of pulmonary diseases other than those typical for end-stage liver diseases were excluded from the study. In this group, 18 patients were men and 4 women, with a mean age of 40.5 years (range 20-67 years). General anesthesia was induced with midazolam (0.1 mg/kg), fentanyl (10 µg/kg), scopolamine (0.6 mg), and vecuronium (0.1 mg/kg), and followed by endotracheal intubation. The anesthesia was maintained by inhalation of isoflurane (0.4-1.0 MAC) and supplements of fentanyl (2-4 µg/kg) and vecuronium (2 mg) as required. An arterial line was put in the left radial artery and a 7-French pulmonary artery catheter was inserted into the right jugular vein to measure blood pressure, central venous pressure, mean pulmonary artery pressure (MPAP) and pulmonary artery occlusion pressure (PAOP). Electrocardiograms, oxygen saturation (SPO₂) and P_{ET}CO₂ were monitored during the whole procedure. In order to sustain body temperature, all patients were positioned on a heating blanket set at 39 °C and all fluid was administered using a warming system (HOTLINE). 500 mg methylprednisolone was administered before reperfusion. The patients were managed using prophylactic mechanical ventilation in the intensive care units for approximately 1-3 days after OLT. The patients were divided into two groups according to whether they had subsequent pulmonary complications: group A (8 patients) with postoperative pulmonary complications; and group B (14 patients) without postoperative pulmonary complications.

Arterial blood samples (3 ml) were taken for gas analysis at the beginning of operation (T₀), clamping and cross-clamping of the inferior cava and portal vein (T₁, T₂), 90 minutes and 3 hours after reperfusion (T₃, T₄) and 24 hours after operation (T₅). Plasma was separated immediately by centrifugation (3000 r/min, 15 minutes) and stored at -20 °C for subsequent determination of plasma TNF-α, IL-6 and IL-8 by enzyme-linked immunoassay (ELISA) as markers of systemic inflammation. Kits were purchased from Biogene, Gemzyme Company, USA. The lower detection limit of the assay was 0.5 pg/ml for TNF-α, 12 pg/ml for IL-6, and 31 pg/ml for IL-8.

All the patients were carefully monitored by regular analysis of blood counts, chest radiographs, sputum cultures, and arterial blood gases during the three days after

operation. The criteria for diagnosis of postoperative pulmonary complication included acute lung injury; the ratio of arterial partial pressure of oxygen (PaO₂) to fraction in spired oxygen (FiO₂) being less than 300 mmHg, and bilateral lung infiltration on chest radiography; adult respiratory distress syndrome (ARDS); PaO₂/FiO₂ < 200 mmHg and bilateral lung infiltration on chest radiography; pneumonia; positive lung infiltration, fever above 38 °C, leucocytes > 10 000 per mm³, and positive sputum cultures.^[19]

The data were expressed as median (M). Variables of the individual groups were compared using the Kruskal-Wallis test while those between the two groups were compared using the Man-Whitney test. General conditions of the two groups was compared by *t* test. The correlation between TNF-α and IL-6 and between TNF-α and IL-8 was measured by Pearson's product-moment correlation coefficient test. A *P* value of less than 0.05 was considered to indicate statistical significance.

Results

General data

Demographic data on age, gender, body weight, duration of operation and anhepatic stage and intraoperative bleeding were compared between the two groups (*P* > 0.05). The pattern of hemodynamic changes was similar in both groups. One patient with ARDS, five patients with acute lung injury and two patients with pneumonia were found in group A, but no patient in group B de-

Table 1. General data on the two groups

| Items | Group A (n=8) | Group B (n=14) |
|--|------------------|-------------------|
| Age (years) | 52±8 | 51±9 |
| Gender (men/women) | 6/2 | 12/2 |
| Body weight (kg) | 60±8 | 58±10 |
| Duration of operation (h) | 5.3±1.2 | 5.1±1.3 |
| Duration of anhepatic stage (min) | 68±14 | 70±12 |
| Bleeding (ml) | 3800±715 | 3950±689 |
| Postoperative respiratory support time (d) | 3.8±1.4 | 1.2±0.5 * |

* : *P* < 0.05 vs group A.

Table 2. Plasma levels of TNF-α, IL-6 and IL-8 (pg/ml)

| Items | Types | T ₀ | T ₁ | T ₂ | T ₃ | T ₄ | T ₅ |
|-------|-------|----------------|----------------|----------------|----------------|----------------|----------------|
| TNF-α | A | 14.5 | 21 | 63.5** | 137** | 33.5** | 15 |
| | B | 12.5 | 16 | 20.5* | 33* | 17.5* | 9 |
| IL-6 | A | 112.5 | 133 | 267.5** | 446** | 210** | 112.5 |
| | B | 111.5 | 126.5 | 183.5* | 343.5* | 172* | 120 |
| IL-8 | A | 20.8 | 26.5 | 686** | 1050** | 406.1** | 15.9 |
| | B | 15.4 | 18.25 | 62.5* | 69.1* | 34* | 18.4 |

* : *P* < 0.05 vs T₀; #: *P* < 0.05 vs group B.

veloped postoperative pulmonary complication. Mean $\text{PaO}_2/\text{FiO}_2$ in group A was lower than that in group B during the three days after operation ($P < 0.05$, Table 1). All the patients developed left-sided pleural effusions after OLT.

Plasma level of TNF- α

The TNF- α concentrations in the two groups increased rapidly at T_2 , peaked at T_3 , decreased rapidly after T_3 until 24 hours after operation. The TNF- α concentrations in group A were higher than those in group B at T_2 , T_3 , and T_4 ($P < 0.05$, Table 2).

Plasma levels of IL-6 and IL-8

The pattern of IL-6 and IL-8 release was similar to that of TNF- α . The IL-6 and IL-8 concentrations in group A were higher than those in group B at T_2 , T_3 , and T_4 ($P < 0.05$, Table 2).

Cytokine kinetics

Correlation was found between TNF- α and IL-6 and between TNF- α and IL-8; the ratio was 0.52 and 0.75, respectively ($P < 0.01$). There was a significant correlation between IL-6 and IL-8 ($r = 0.48$, $P < 0.01$).

Discussion

Hepatic ischemic-reperfusion injury is thought to be a complex pathological course, in which many factors participate. After cold ischemic storage and subsequent reperfusion, increased vasoactive compounds released from donor liver and gut may lead to tissue damage and endothelial defect, and increased release of oxygenated free radicals can directly damage donor liver.

TNF- α is now implicated as a mediator of various physiologic and pathophysiologic events including inflammation as well as differentiation and apoptosis of cell survival growth. In agreement with previous studies, increased plasma TNF- α levels were observed in all patients after reperfusion in the present study. They increased rapidly at T_2 , peaked at T_3 , and maintained at a high level till 24 hours after operation.^[17] The liver may be a big reservoir for Kupffer cells, which are one of sources of TNF- α . Kupffer cells can be activated while donor liver is stored for over 4 hours in ice-cold Euro-Collins solution.^[20,21] The inferior cava and portal vein were clamped for about 60 minutes during OLT without veno-venous bypass, resulting in mesenteric venous congestion and increased intestinal permeability. Then bacteria/endotoxin were translocated from the gut lumen to the portal circulation. At the time of reperfusion, endotoxin in the portal vein increased rapidly and stimulated these activated Kupffer cells to synthesize, express and

release TNF- α .^[17,22] Being responsible for the induction of IL-1 which can induce IL-6 and IL-8, TNF- α can form a complex multiple level cytokines network in the body.^[23] This concept is confirmed by this study because the pattern of IL-6, IL-8 release was similar to that of TNF- α .

Animal models of hepatic ischemic-reperfusion injury have demonstrated increased expression of TNF- α after reperfusion^[24] and higher concentration of TNF- α also correlate with severe lung injury.^[17] This view was confirmed by our finding in this study that the TNF- α concentrations in group A were higher than those in group B at T_2 , T_3 , and T_4 . Moreover, the IL-6 and IL-8 concentrations in group A were higher than those in group B at T_2 , T_3 , and T_4 . This indicated that increased release of IL-6 and IL-8 is associated with subsequent pulmonary impairment and that patients with higher levels of intraoperative IL-6 and IL-8 would develop severe pulmonary complications. TNF- α , IL-6 and IL-8 as proinflammatory cytokines attack and activate polymorphonuclear neutrophil granulocytes mutually, then up-regulate cell surface expression of adhesive molecules on neutrophils including CD11/CD18, which allow for tight adherence to the endothelium. At the same time, these cytokines activate the vascular endothelium, increasing the cell surface expression of adhesive molecules including intracellular adhesive molecule 1 (ICAM-1), with recruitment and adherence of neutrophils. Activation of these neutrophils can lead to endothelial injury, capillary leak and impairment of gas exchange.^[23]

Oxygenation index can be used as an indication for pulmonary complications after OLT.^[24] In this study, mean $\text{PaO}_2/\text{FiO}_2$ was lower in group A than in group B during the three days after operation, which confirmed that significantly increased levels of TNF- α , IL-6 and IL-8 after reperfusion were associated with subsequent lung injury.

Our findings suggest that patients with ample release of TNF- α , IL-6 and IL-8 after cross-clamping of the inferior cava and portal vein easily develop postoperative complications. Pretreatment with methylprednisolone^[23] and anti-TNF- α antibody^[24] before reperfusion can markedly inhibit release of TNF- α , IL-6 and IL-8, lower recruitment and adherence of neutrophils, and reduce the incidence of pulmonary complications.

Competing 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.

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Received July 11, 2003

Accepted after revision December 2, 2003