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Ischemic Reperfusion Injury–Induced Oxidative Stress and Pro-inflammatory Mediators in Liver Transplantation Recipients

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ABSTRACT

Objective. Liver ischemic reperfusion injury is harmful to transplant recipients, and is associated with postoperative morbidity and mortality. Our study was designed to investigate the oxidative stress and pro-inflammatory mediators in liver transplant recipients.

Methods. We prospectively analyzed 14 recipients who underwent liver transplantation by measuring their blood levels of malondialdehyde (MDA) and cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6, at nine time points perioperatively. We also evaluated the correlations between oxidative stress (MDA levels) and the characteristics of the recipient or the donated graft.

Results. These parameters significantly increased from 1 minute before reperfusion, and the values peaked within 3 to 30 minutes after reperfusion. On the time point at 5 minutes after reperfusion, the MDA levels which were the highest in the recipients correlated with the values of preoperative direct/and total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), model for end-stage liver disease (MELD) score, international normalized ratio (INR), and surgical blood loss.

Conclusion. The levels of MDA, TNF-α, IL-1β, and IL-6 greatly increased with the ischemic reperfusion insult. Recipients with higher values of preoperative direct/and total bilirubin, AST, ALT, MELD score, INR, and surgical blood loss tended to have higher levels of MDA and may suffer more injury from this insult.

AS TRANSPLANTATION surgery evolves, orthotopic liver transplantation (OLT) is becoming an effective treatment for patients with liver failure or hepatocellular carcinoma. In this complex process, it is reoxygenation of hypoxic tissue that causes harmful ischemic reperfusion injury (IRI). Liver IRI is associated with postoperative graft dysfunction, transplant failures or rejections, and other morbidity.

Reactive oxygen species (ROS) abruptly increase when the tissues or organs are ischemic and then reperfuse and cause generally inflammatory progressions. ROS play a critical role in this pathophysiological process, and they could lead to microvascular dysfunction and parenchymal injury in the allograft [1]. As in liver IRI transplant surgery, the primary sources of ROS are cytosolic xanthine oxidase, mitochondria, Kupffer cells, and adherent polymorphonuclear granulocytes [2]. Oxidative stress-induced liver injury includes lipid peroxidation of the cell, affecting the mitochondrial membrane potential to trigger necrosis, and causing apoptotic cell death. Malondialdehyde (MDA) is one of the intermediate metabolites from ROS-induced lipid peroxidation. Oxidative stress can be indirectly represented by measuring MDA levels.

Many studies have shown that the inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin-1β [IL-1β], and interleukin-6 [IL-6]) are involved in systemic inflammation, inducing apoptotic cell death, inflammation, and IRI [1,3,4]. The oxidative stress has been documented, but the

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0041-1345/14/$–see front matter
http://dx.doi.org/10.1016/j.transproceed.2014.01.009

IRI-induced pro-inflammatory mediators in liver transplantation recipients are far from being evaluated completely.

Our study was designed to investigate intraoperative changes of oxidative stress by measuring MDA levels and the productions of TNF-α, IL-1β, and IL-6 associated with IRI in human recipients of OLT.

MATERIALS AND METHODS

Study Design and Patients

Our prospective study was approved by the Institutional Review Board of Chang Gung Memorial Hospital. All recipients were well informed and completed a valid consent before the day of surgery. Fourteen recipients (42 to 67 years old) who required relative OLTs were enrolled into this study without any exclusion criteria. Exclusion criteria included patients who had recently suffered from sepsis, hepatoencephalopathy, or hepatorenal syndrome, and those who used cigarettes or antioxidants.

For blood sampling, an arterial catheter was placed in the right radial artery before induction of anesthesia. After the first blood sampling as a baseline measurement, general anesthesia was conducted with midazolam at 0.2 to 0.3 mg/kg, rocuronium at 0.5 to 0.6 mg/kg, and fentanyl at 3 μg/kg intravenously. Every recipient was intubated with an endotracheal tube and was under controlled ventilation with tidal volume at 10 mL/kg, rate at 10 to 12 breaths/min. Respiratory end-tidal CO_2 was maintained within 30 to 35 mm Hg. Isoflurane 1.2 to 1.6% (1–1.3 minimum alveolar anesthetic concentration) with 100% oxygen was inhaled for anesthesia maintenance. Muscle relaxation was completed by intravenously administrating bolus rocuronium (0.2 mg/kg) in regular intervals. Mean arterial blood pressure was monitored by connecting a pressure kit to the left radial artery and was maintained at approximately 60 to 90 mm Hg.

All liver grafts were donated by patients' families or relatives. The liver grafts were flushed with iced-cold histidine-tryptophan-ketoglutarate solution into the portal vein and were then preserved in 500 mL same solution before the transplant surgeries. The time point was defined as starting point of ischemia when vessels of the donated liver graft were clamped. The ischemic time was measured from this start point until graft reperfusion.

Five milliliters of blood were drawn at nine predefined time points: T0 (baseline), before the induction of anesthesia; T1, 1 hour after surgical incision; T2: 1 minute before reperfusion; T3, 30 seconds after reperfusion; and T4-8, 1, 3, 5, 30, and 60 minutes after reperfusion.

The blood samples were kept in 4°C ice water right after it was withdrawn. We separated the plasma by centrifugation (3500 rpm/10 min) immediately and stored it in a refrigerator at −80°C until analysis.

We evaluated plasma levels of MDA by the method adopted in our previous study [5]. The MDA level was analyzed by a thiobarbituric acid assay. We measured the levels of thiobarbituric acid reactive substances by reading the absorbance at 535 nm with a spectrophotometer, and the concentration was expressed as millimoles per gram protein (mM/g protein). The plasma levels of TNF-α, IL-1β, and IL-6 were analyzed by enzyme-linked immunosorbent assay and the values were shown as picograms per gram of protein (pg/g protein).

Statistical Analysis

Data were analyzed with SPSS version 15 (SPSS Inc, Chicago, Ill, United States). The levels of MDA and cytokines (TNF-α, IL-1β, and IL-6) were analyzed and compared using the Wilcoxon signed ranks test. The relationships between MDA level at T6 and the characteristics of the recipient or the donated graft were analyzed by using nonparametric correlations (Spearman's rho tests). These characteristics included the ischemic time of the graft, the length of the anhepatic phase, the time in surgery before reperfusion (dissecting phase), the weight of the graft, the ages of the donor and recipient, systemic vascular resistance at T1, preoperative international normalized ratio (INR), platelet count, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, model for end-stage liver disease (MELD) score, blood loss, and volume of drained ascites. A value of $P < .05$ was considered to be statistically significant.

RESULTS

Fourteen patients were enrolled into this study. Eight recipients were victims of liver cirrhosis, 4 had hepatocellular carcinoma, and the other 2 had both diseases (Table 1). Plasma concentrations of MDA increased slightly at T1 without statistical significance compared with T0. The values reached peak levels at T6. The MDA level then decreased to 86.5 ± 52.9 mM/g protein at T8. The levels of MDA at T2-8 were all significantly higher than at T0 (Table 2).

Plasma concentrations of TNF-α also increased slightly at T1 without statistical significance compared with T0, and then peaked at T5. The levels of TNF-α at T2-8 were significantly higher than at T0. Plasma concentrations of IL-1β increased slightly at T1 without statistical significance compared with T0. The levels of IL-1β at T2-8 were significantly higher than at T0. The levels of IL-6 at T2-8 were significantly higher than at T0 (Table 2).

Table 1. Preoperative Demographic Features and Intraoperative Events

Variables	Mean
Age (y)	55.07 ± 9.06
Height (cm)	162.21 ± 9.85
Weight (kg)	60.75 ± 13.03
Sex ratio (M/F)	9/5
MELD scores	16.64 ± 10.95
Pre-op INR	1.64 ± 0.42
Pre-op total bilirubin	6.92 ± 12.22
Pre-op direct bilirubin	3.54 ± 6.23
Pre-op AST	67.29 ± 40.05
Pre-op ALT	45.43 ± 18.90
Pre-op platelet number (×1000)	46.00 ± 21.39
Ischemic time (min)	85.15 ± 22.08
Graft weight (g)	533.46 ± 207.40
Blood loss (mL)	1890.00 ± 1314.10
Ascites (mL)	1500.00 ± 1952.12
Age of donor (y)	33.21 ± 9.33
Dosage of dopamine (mg)	26.96 ± 17.95

Data are presented as mean ± standard deviation.

Abbreviations: MELD, model for end-stage liver disease; INR, international normalized ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase.