



Changes of nitric oxide and endothelin, thromboxane A₂ and prostaglandin in cirrhotic patients undergoing liver transplantation

Zi-Qing Hei, He-Qing Huang, Chen-Fang Luo, Shang-Rong Li, Gang-Jian Luo

Zi-Qing Hei, Chen-Fang Luo, Shang-Rong Li, Gang-Jian Luo, Department of Anesthesiology, Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China
He-Qing Huang, School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510080, Guangdong Province, China
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Co-first-authors: He-Qing Huang

Correspondence to: Zi-Qing Hei, Department of Anesthesiology, Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China. heiziqing@sina.com

Telephone: +86-20-85516867-3132

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Abstract

AIM: To investigate the perioperative changes of nitric oxide (NO) and endothelin (ET), thromboxane A₂ (TXA₂) and prostaglandin (PGI₂) during liver transplantation in end-stage liver disease patients.

METHODS: Twenty-seven patients with end-stage cirrhosis undergoing liver transplantation were enrolled in this prospective study. Blood samples were obtained from superior vena at five different surgical stages. Plasma concentrations of nitrate and nitrite were determined to reflect plasma NO levels. Plasma levels of ET-1, 6-keto-PGF₁ alpha and thromboxane B₂ (TXB₂), the latter two being stable metabolites of PGI₂ and TXA₂ respectively, were measured.

RESULTS: The NO level decreased significantly after vascular cross-clamping and increased significantly at 30 min after reperfusion. While the ET levels at 30 min after clamping and after reperfusion were significantly elevated. The ratio of NO/ET decreased significantly at 30 min after vascular cross-clamping and at the end of surgery. The PGI₂ level and the TXA₂ during liver transplantation were significantly higher than the baseline level, but the ratio of TXA₂/PGI₂ decreased significantly at 30 min after clamping.

CONCLUSION: NO/ET and TXA₂/PGI₂ change during liver transplantation. Although the precise mechanism remains unknown, they may play a role in the pathobiology of a variety of liver transplant-relevant processes.

INTRODUCTION

Liver transplantation is becoming a more and more common treatment method of end-stage liver disease. The hemodynamic alterations occurring after graft reperfusion and the subsequent complications may result from the changes of some biotic activators^[1]. Nitric oxide (NO) and endothelin (ET), thromboxane A₂ (TXA₂) and prostaglandin (PGI₂) are two groups of important vasoactive substance. The clinical and animal experiment documented that the two groups of substance are involved in a variety of liver transplant-relevant processes, including ischemia-reperfusion injury, acute cellular rejection, and circulatory changes characteristic of advanced liver disease^[2]. However, there is considerable controversy in the perioperative plasma concentrations of the two groups of substance and the precise mechanism of these changes is not fully known.

This study monitored blood levels of NO and ET, TXA₂ and PGI₂ at different time points perioperatively in cirrhotic patients undergoing liver transplantation.

MATERIALS AND METHODS

Patients

During a 10-mo period, all patients with end-stage cirrhosis undergoing liver transplantation were enrolled in this prospective study. With the approval of the institutional ethics committee from Sun Yet-Sen University, informed consent was obtained from patient before each assessment. We excluded patients receiving liver transplantation more than one time and patients losing blood more than 3500 mL during operation.

Anesthesia was induced with midazolam (0.05-0.1 mg/kg), propofol (1-2 mg/kg) and fentanyl (5 µg/kg) intravenously (IV) and neuromuscular blockade was accomplished with nocurium (0.1 mg/kg). Mechanical ventilation was performed with O₂, using a tidal volume necessary to maintain ET/CO₂ tension between 4-4.66 kPa (30-35 mmHg). Anesthesia was maintained with both isoflurane at a concentration of 1.0%-3.5% (inhalation) and intermittent fentanyl (50-100 µg, IV). After induction of anesthesia, a continuous dopamine infusion (2-6 µg/kg per min) was administered. Blood samples were obtained from the superior vena cava at five different surgical stages: induction of anesthesia (T1), 60 min after beginning of operation (T2), 30 min after vascular cross-clamping (T3), 30 min postreperfusion of new liver (T4) and at the end of surgery (T5). Plasma concentrations of nitrate and nitrite, two metabolites of NO, were determined to reflect plasma NO levels. Plasma ET-1 levels were measured by radio-immunoassay (RIA) and systemic 6-keto-PGF₁α and TXB₂, stable metabolites of PGI₂ and TXA₂ respectively, were measured by RIA also.

Detection of NO

Two milliliters of superior vena cava blood were collected and placed in ice-cooled polypropylene tubes. These samples were immediately centrifuged at 3000 r/min for 10 min and stored at -40°C for late assay. Test kits (Science and Technology Development Center of The General Hospital of PLA, Beijing, China) for NO analysis were used. The optical density value was read in a spectrophotometer set at 546 nm. The NO numeric value was calculated based on a standard and empty tube according to the equation. The calculated results were expressed as µmol/L.

Detection of ET

Two milliliters of superior vena cava blood were collected and placed in ice-cooled polypropylene tubes containing 40 µL aprotinin and 30 µL EDTA. These samples were immediately centrifuged at 3000 r/min for 10 min. Collected plasma was stored at -40°C until batched assays were performed. Plasma ET levels were evaluated with ET detection kit (Science and Technology Development Center of The General Hospital of PLA, Beijing, China) according to the manufacturer's instructions.

Detection of TXA₂ and PGI₂

Three milliliters of superior vena cava blood were collected with a five-milliliter syringe containing 200 µL EDTA·2Na, mixed fully and injected into a tube. These tubes were immediately centrifuged at 3500 r/min for 15 min. Collected plasma was stored at -40°C until use. Detection of the level of TXA₂ and PGI₂ was performed according to the manufacturer's instructions (Science and Technology Development Center of The General Hospital of PLA, Beijing, China).

Statistical analysis

Data are shown as mean ± SD. Data of the various time points were compared using ANOVA analysis (SPSS statistical package, version 11.0). Significant difference was accepted at $P < 0.05$.

Table 1 Changes of NO₂/NO₃ and ET levels in plasma during liver transplantation (mean ± SD, $n = 27$)

	NO ₂ /NO ₃ (µmol/L)	ET (ng/L)	NO/ET
T1	27.45 ± 5.70	61.21 ± 25.38	0.54 ± 0.25
T2	35.19 ± 7.72 ^a	74.84 ± 29.22	0.49 ± 0.25
T3	25.64 ± 5.64 ^c	110.61 ± 24.65 ^{a,c}	0.24 ± 0.07 ^{a,c}
T4	35.30 ± 11.23 ^{a,e}	114.00 ± 26.73 ^{a,c}	0.32 ± 0.13 ^a
T5	33.86 ± 8.94 ^c	116.92 ± 27.08 ^{a,c}	0.28 ± 0.07 ^{a,c}

^a $P < 0.05$ vs T1; ^c $P < 0.05$ vs T2; ^e $P < 0.05$ vs T3. T1: induction of anesthesia; T2: 60 min after operation beginning; T3: 30 min after vascular cross-clamping; T4: 30 min postreperfusion of new liver; T5: the end of operation.

Table 2 Changes of TXB₂ and 6-keto-PGF₁α levels in plasma during liver transplantation (mean ± SD, $n = 27$)

	6-keto-PGF ₁ α (ng/L)	TXB ₂ (ng/L)	TXB ₂ /6-keto-PGF ₁ α
T1	152.94 ± 68.67	118.27 ± 63.99	0.89 ± 0.37
T2	932.71 ± 25.66 ^a	297.45 ± 127.13 ^a	0.48 ± 0.25
T3	895.83 ± 300.71 ^a	265.15 ± 127.34 ^a	0.33 ± 0.14 ^a
T4	620.94 ± 282.41 ^{a,c,e}	271.26 ± 140.48 ^a	0.49 ± 0.21 ^e
T5	591.12 ± 336.57 ^{a,c,e}	208.32 ± 90.98 ^{a,c,e}	0.43 ± 0.20

^a $P < 0.05$ vs T1; ^c $P < 0.05$ vs T2; ^e $P < 0.05$ vs T3. T1: induction of anesthesia; T2: 60 min after operation beginning; T3: 30 min after vascular cross-clamping; T4: 30 min postreperfusion of new liver; T5: the end of operation.

RESULTS

A total of 27 patients (81% men, age 49 ± 11 year, BSA 1.72 ± 0.15) were enrolled in this study. Among these patients, 15 had hepatitis B virus associated cirrhosis, 12 had cirrhosis and liver cancer.

The changes of nitrate, nitrite and ET levels in plasma during liver transplantation are shown in Table 1. The mean baseline value of NO (reflected by nitrate and nitrite) was 27.45 ± 5.70 µmol/L. The NO level decreased significantly after vascular cross-clamping, from 35.19 ± 7.72 µmol/L to 25.64 ± 5.64 µmol/L ($P < 0.05$). At 30 min after reperfusion, a significant elevation of NO was found, from 25.64 ± 5.64 µmol/L to 35.30 ± 11.23 µmol/L ($P < 0.05$).

The baseline ET concentration was 61.21 ± 25.38 ng/L. Compared with the baseline level, the ET levels at 30 min after clamping and after reperfusion were significantly elevated (110.61 ± 24.65 ng/L, 114.00 ± 26.73 ng/L respectively).

The changes of TXB₂ and 6-keto-PGF₁α levels in plasma during liver transplantation are shown in Table 2. The prostaglandin level and the TXA₂ level presented the same changes. The concentrations at every time point were significantly higher than the baseline level. Peak levels of prostaglandin were 932.71 ± 25.66 ng/L and 895.83 ± 300.71 ng/L, detected at 60 min after operation and 30 min after vascular cross-clamping respectively.

The mean baseline value of NO/ET was 0.54 ± 0.25. The ratio decreased significantly at 30 min after vascular cross-clamping and at the end of surgery. The mean

baseline value of TXA₂/PGI₂ was 0.89 ± 0.37 , the ratio decreased significantly at 30 min after clamping. Compared with the value at 30 min after clamping, the ratio increased significantly at 30 min after reperfusion, from 0.33 ± 0.14 to 0.49 ± 0.21 .

DISCUSSION

In this study we measured systemic NO and ET, TXA₂ and PGI₂ at five different surgical stages: basal, hepatectomy, anhepatic, 30 min after graft reperfusion and the end of surgery. Overall results showed that the levels of these two groups of vasoactive substances changed at each stage during liver transplantation. Previous studies have demonstrated that NO and ET, TXA₂ and PGI₂ play an important role in the pathobiology of ischemia-reperfusion injury and postreperfusion syndrome^[3,4]. It suggests that disturbed balance between these vasodilators and vasoconstrictors may contribute to some liver transplantation-relevant syndromes.

NO and ET are the most important local vasodilator and vasoconstrictor respectively. They seem to play a role in almost every organ and tissue. However, there is considerable confusion in understanding their roles. Some researches suggest that the important factors in determining the beneficial versus harmful effects of NO are the amount, duration, and site of NO production^[5]. Ovadia *et al* demonstrated that the fetal pulmonary vasoconstriction after acute constriction of the ductus arteriosus is mediated by NO/ET-1 interactions^[6]. Shirakami *et al* reported that the plasma ET level was increased before transplantation compared with that of healthy children, but decreased during the anhepatic phase^[7]. It increased again after reperfusion and remained at high level in the early postoperative period. These suggest that ET production in the cirrhotic liver is augmented and ET plays some role in circulatory regulation during the perioperative period of pediatric liver transplantation. In the present study, although both NO and ET increased after graft reperfusion, the ratio of them was decreased. It suggested that the imbalance of NO and ET level may participate in the pathophysiology of systemic and local circulation disorders.

Many data about the relationship between another group of vasoactive substances, PGI₂/TXA₂ and hemodynamics have been reported^[8,9]. TXA₂ is both a vasoconstrictor and a potent stimulus for platelet aggregation. Its effect is antagonized by prostacyclin, which is released by vascular endothelial cells. Prostacyclin exerts a variety of effects on the cardiovascular system, including a decrease in blood pressure associated with a decrease in systemic vascular resistance. In clinical liver transplantation, Khoury *et al*^[10] demonstrated 60% of patients undergoing orthotopic liver transplantation accumulated prostacyclin in the portal vein, which could be one of the causes of hypotension seen at reperfusion of the donor liver. As previously demonstrated, we found that the baseline levels of PGI₂ and TXA₂ in our patients

were higher than normal values. It was most likely due to the decreased metabolism of them in patients with end-stage liver disease. In addition, we found PGI₂ and TXA₂ had the same changes during liver transplantation. The levels of PGI₂ and TXA₂ after reperfusion were elevated compared with the baseline level. However, the ratio of TXA₂ and PGI₂ was significantly lower than that of pre-reperfusion. This indicated that the disorder of TXA₂ and PGI₂ might also be involved in the circulation disorders during orthotopic liver transplantation.

In summary, two groups of endogenous vasoactive substance, NO/ET and TXA₂/PGI₂, are changed during liver transplantation. Although the precise mechanism remains unknown, they may play a role in the pathobiology of a variety of liver transplant-relevant processes.

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