Plasma levels of endothelin-1 in patients with the hepatorenal syndrome after successful liver transplantation

**Abstract** The hepatorenal syndrome (HRS) is characterized by renal vasoconstriction leading to deterioration of renal function in patients with liver disease. A possible role of endothelin-1 (ET-1) in the pathogenesis of HRS has been suggested, but a correlation between ET-1 plasma levels and the development of HRS as well as the recovery from HRS following OLT has not been shown yet. We performed longitudinal measurements of ET-1 plasma levels in four groups of patients, 5 patients with HRS before and after orthotopic liver transplantation (OLT), 10 patients without HRS undergoing OLT, 20 patients with chronic renal failure but without liver disease, and 12 healthy controls. Before OLT, plasma levels of ET-1 were higher in patients with HRS (19.5 ± 8.6 ng/l, P < 0.001; n = 5) compared to patients without HRS (4.9 ± 1.1 ng/l; n = 10), normals (1.2 ± 0.18 ng/l; n = 12), and patients with chronic renal failure (2.4 ± 0.4 ng/l; n = 20). Patients with HRS compared to patients without HRS had higher levels for creatinine clearance (2.42 ± 0.6 vs. 0.89 ± 0.05 mg/dl, P < 0.05), creatinine clearance (107 ± 9 ml/min vs. 44.6 ± 5.5 ml/min, P < 0.001), and bilirubin (11.4 ± 3.8 vs. 3.7 ± 1 mg/dl, P < 0.05) before OLT. Within one week after OLT, there was a rapid decrease in ET-1 levels in patients with HRS while creatinine and bilirubin levels decreased slower. Regression analysis revealed a weak correlation between serum creatinine and ET-1 (r = 0.192, P = 0.04) and a significant correlation between serum bilirubin and ET-1 (r = 0.395, P < 0.001). The means of the ET-1 levels decreases rapidly with improvement of liver function after OLT. Levels of ET-1 correlate with excretory liver function assessed by bilirubin. The fall in ET-1 levels preceding improvement of renal function further strengthens the concept of ET-1 being a causative factor in HRS.

**Key words** Endothelin-1 · Hepatorenal syndrome · Liver transplantation · Renal failure

**Abbreviations** HRS Hepatorenal syndrome · ET-1 Endothelin-1 · OLT Orthotopic liver transplantation

**Introduction**

The HRS is characterized by intense renal vasoconstriction leading to renal dysfunction in patients with advanced liver failure [5]. The prevalence of HRS in liver recipients before transplantation is approximately 10% [9]. The pure functional nature of HRS is supported by the observation that kidneys from brain-dead patients with HRS can be successfully transplanted and function well in patients with endstage renal disease [18]. More-
over, after successful OLT of patients with HRS, urine volume increases within 3 days after OLT, and renal function improves although the glomerular filtration rate remains lower than in patients without HRS [14].

The pathogenesis of HRS is not fully understood [5]. Portal hypertension is accompanied by splanchic and peripheral vasodilatation. Fluid losses into the peritoneal cavity may lead to reduced effective arterial blood volume, triggering the renin angiotensin system, the sympathetic nervous system and the secretion of antidiuretic hormone with the consequence of water and salt retention by the kidneys. However, unlike in prerenal azotemia, in HRS, renal function cannot be fully restored by the administration of fluids. Because of the observation of peripheral vasodilatation in the presence of renal vasoconstriction, it was suggested that the Endothelins may play a pathogenic role in HRS [21]. The Endothelin family consists of three isoforms produced as large precursors preproendothelin that are cleaved by Endothelin-Converting-Enzyme. The renal vasculature is uniquely sensitive to ET, especially to ET-1. Infusion of 2.5 ng/kg per min ET-1 into normal volunteers leads to dramatic decreases in renal function but has only modest effects on systemic blood pressure [27]. Moore and colleagues reported increased plasma levels of ET-1 in patients with chronic liver diseases compared to normals [21]. Patients with liver disease and HRS had twice as high plasma levels of ET-1 compared to patients without HRS, indicating that ET-1 has a pathogenic role in HRS. However, an association does not prove causal relationship, and the transversal nature of this study has been debated [1] since it did not follow patients with HRS after improvement of liver function, for example by liver transplantation. The present study is the first one providing longitudinal measurements of ET-1 in individual patients. If ET-1 is a pathogenic factor in HRS, plasma levels of ET-1 should decrease and renal function should improve after improvement of liver function. To test this hypothesis, we performed longitudinal measurements of ET-1 plasma levels in patients with HRS undergoing OLT.

**Methods**

**Study subjects**

We studied two groups of patients with liver disease awaiting OLT. All patients except for one had liver failure stage Child B–C. Group 1 (n = 10) consisted of patients with serum-creatinine values <1.2 mg/dl before transplantation. The cause of liver failure was hepatitis C (n = 4), hepatitis B (n = 1), M. Wilson (n = 1), alcoholic cirrhosis (n = 1), autoimmune hepatitis (n = 1), primary sclerosing hepatitis (n = 1) and Katskin tumor (n = 1). Two of these patients developed acute renal failure after OLT and were excluded from the analysis.

Group 2 consisted of 5 patients with serum-creatinine values >1.2 mg/dl before transplantation. Serum-creatinine increased within the 4 weeks before OLT in 3 patients and was steadily elevated for at least 2 months before OLT in two patients. The cause of liver failure was alcoholic cirrhosis (n = 2), hepatitis B (n = 1), autoimmune hepatitis (n = 1), and unknown (n = 1). Renal ultrasound revealed normal kidney size in all patients. In Group 2, the central venous pressure at the start of transplantation was 12 ± 2.3 mm Hg.

Group 3 consisted of 20 patients from the nephrology ward with different degrees of chronic renal insufficiency but without liver disease.

Different immunosuppressive protocols were used during the course of the study, all of them included steroids and either cyclosporine A (CyA) or tacrolimus.

As controls, 12 healthy subjects from the medical staff were studied.

**Sample collection**

For measurement of ET-1, blood samples were drawn between 8 and 9 a.m. In groups 1 and 2, blood was drawn before OLT and at least at weekly intervals up to 4 weeks after OLT. In groups 3 and 4, blood was drawn once. 10 ml of blood was drawn into tubes containing EDTA (Sigma-Aldrich GmbH, Deisenhofen, Germany, final concentration 1 mg/ml), chilled on ice, and immediately centrifuged at 4 °C. Plasma was obtained and stored at –70 °C until extraction.

For determination of creatinine and bilirubin in groups 1 and 2, blood was drawn immediately before OLT and at least at weekly intervals up to 4 weeks after OLT. After 4 weeks, patients were followed in the outpatient clinic at 3 and 6 months after OLT. Serum creatinine and bilirubin were determined as routine parameters in our central laboratory. GFR was calculated by the formula of Cockcroft and Gault: 

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\text{Cr-Clearance} = (140-\text{age}) \times \text{kg BW} / (\text{mg/dl serum-creatinine} \times 72).
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**Measurement of ET-1**

ET-1 was extracted from plasma by a modification of the method of Moore et al. [21]. C18 SepPak columns (Waters Corp., Milford, MA) were rinsed with 5 ml acetonitrile/0.1% trifluoroic acid followed by 5 ml methanol/0.1% trifluoroic acid and destilled water/0.1% trifluoroic acid. After application of 3 ml of plasma, columns were rinsed with destilled water/0.1% trifluoroic acid and 40% methanol/0.1% trifluoroic acid. Endothelin was eluted with 3 ml 70% methanol/0.1% trifluoroic acid/0.01% triton X (Sigma). The eluate was dried under vacuum and resuspended in 500 µl of RIA buffer. ET-1 was measured in duplicate by RIA (Rik 6001, Peninsula Laboratories Inc., Belmont, CA) with a sensitivity of 1 pg/tube. Because 100 µl of the 500 µl RIA buffer used to resuspend 3 ml of plasma sample was added per tube, the sensitivity was 0.6 pg/ml plasma. The cross-reactivity of the RIA as outlined by the manufacturer is 17% for Big-ET-1, 7% for ET-2, and 7% for ET-3.

**Statistical analysis**

ET-1 levels between groups 1–4 were analyzed by ANOVA followed by Bonferroni/Dunn analysis (StatView, Abacus Concepts Inc.). Comparisons of creatinine, calculated creatinine clearance, bilirubin and ET-1 between group 1 and 2 were performed by unpaired t-test. In case of multiple measurements within weekly intervals after OLT, data were averaged per week. Levels for creatine...