Transpl Int (1994) 7 [Suppl 1]: S144–S149

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Intraoperative evaluation of big-endothelin plasma levels during liver transplantation in different vascular compartments

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Abstract Endothelin-1 (ET) is derived from its precursor big-ET, secreted by endothelial cells of multiple origin. The role of ET peptides in the physiological responses after orthotopic liver transplantation (OLT) was investigated. Venous big-ET plasma levels were analysed by RIA in 28 patients before and after OLT. Samples for analysis were taken intraoperatively from 12 patients from the caval, portal and hepatic veins and the radial artery at multiple time points. Highest caval levels were found during the anhepatic period and 60 min after reperfusion, followed by a drop and subsequent increase postoperatively. Highest levels in the hepatic and portal veins were detected during explanation and reperfusion. A different pattern was found in the radial artery. Values during rejection and infection were elevated compared with preoperative and postoperative levels. The heterogeneity of the kinetics points to different sites of ET generation, including liver and splanchnic circulation. It suggests a predominant paracrine secretion mode of ET peptides with various stimuli involved. Big-ET levels could reflect endothelial cell damage, as big-ET is generated intracellularly and biological activity is rather weak.

Key words Endothelin
Big-endothelin · Orthotopic liver transplantation

Introduction

Endothelin-1 (ET) is a potent vasoconstrictor peptide. Activities in nonvascular tissue include positive ino- and chronotropic action, stimulation of atrial natriuretic factor release, inhibition of renin release in the kidney, induction of aldosterone production and glycogenolysis in hepatocytes, mitogenic actions and modification of neuronal excitation [30]. ET seems to be secreted mostly abluminally by vascular endothelial cells of multiple origin [13, 30, 31]. Since low ET levels are present in plasma from several species, a systemic hormonal function has been questioned [4, 13]. Big-ET, the precursor of ET, is derived from a prepropeptide by endoproteolytic liberation. Active ET is formed by intracellular cleavage of a C-terminal fragment from the intermediate, induced by a putative converting enzyme [5, 31]. Increased plasma concentrations have been detected in chronic liver disease [1, 11, 15, 16, 27] and after orthotopic liver transplantation (OLT) [9, 11, 27]. Sites of ET/big-ET generation are uncertain. OLT often leads to changes in cardiovascular function and volume homeostasis characterized by rising arterial pressure, renal vasoconstriction and a fall in GFR [19, 27], resembling effects after experimental i. v. administration of ET [3, 8, 13, 15, 17, 23, 29].

The role of ET peptides in responses after OLT were investigated. Identification of sources and time points of big-ET generation during OLT may help to explain and to interprete postoperative plasma kinetics. A high degree of ET-1 decay has been reported after short periods of deep freezing of blood samples. Big-ET seems to be less affected by freezing and storing at −20 °C [22] and a sufficiently tight correlation with circulating ET appears proven.

Materials and methods

Intraoperative and perioperative samples were taken from 12 selected patients from various vessels during different phases of uncomplicated and stable OLT for the determination of big-ET levels in plasma. Samples from central caval vein and radial artery were taken by the anaesthetist, and simultaneously by direct puncture from the portal and hepatic veins by the surgeon at predefined times: before OLT, at the end of the explantation period, at the end of the anhepatic period, 3, 60 and 120 min after reperfusion, 12 h after OLT and on days 2– 5 after OLT. (No samples from the portal and hepatic veins could be taken during the anhepatic period because of clamping of the hepatic vessels and portosystemic shunting). No arterial samples were taken before and after OLT. No surgical complications such as major blood loss, extensive transfusion, marked prolongation of the operation time or periods of severe arterial hypotension led to exclusion from the intraoperative sampling procedure. Informed consent for blood sampling was obtained prior to surgery.

Big-ET plasma levels from the central caval or cubital veins were analysed in 28 patients before and during a prolonged postoperative course (up to 53 days). Postoperative samples were grouped according to clinical criteria into early postoperative period, stable graft function (uncomplicated clinical course, $n = 19$), episodes of acute rejection (proved by histology, $n = 10$) and infection (biliary peritonitis, $n = 3$; bacterial pneumonia, $n = 2$; bacterial cholangitis, $n = 2$, *Candida* sepsis, $n = 1$; intra-abdominal abscess, $n = 2$).

Samples were immediately stored in EDTA-coated polystyrene tubes and frozen at −20 °C until analysis. Big-ET plasma concentrations were analysed in duplicate using a RIA (Big-ET-RIA-BI-10210; Biomedica, Vienna). In brief [4], big-ET was extracted from plasma by a solid-phase extraction step on reverse phase silica (Sep-Pak C-18 cartidges; Waters/Millipore). The eluant was dried and concentrated by a stream of nitrogen (37 °C). Calibration standards (big-ET lyophilized), HPLC-purified ^{125}I-labelled big-ET-tracer, highly specific and sensitive rabbit anti-(porcine)-big-ET antibodies and plasma extracts were dissolved in sodium phosphate buffer. Separation of the bound big-ET fraction was achieved by a second antibody precipitation after preincubation at room temperature (anti-rabbit-Ig immunoprecipitating reagent; Sorin/Biomedica Saluggia). Cross-reactivity to ET 1–3 was < 1%. The sensitivity of the assay was 0.8 pg/ml. The intra-assay and interassay coefficients of variation were 12.8% and 17.2%, respectively.

Indications for OLT were postinfectious cirrhosis ($n = 5$), alcoholic cirrhosis ($n = 7$), hepato/cholangiocellular carcinoma ($n = 8$), thorotrastoma ($n = 1$), fulminant hepatic failure ($n = 5$) and primary biliary sclerosis ($n = 2$). The mean age of the patients at the time of OLT was 45.4 ± 15 years. Standardized immunosuppression started after OLT comprised methylprednisolone (100 mg/day on a tapering schedule), azathioprine (2 mg/kg per day), CsA (beginning

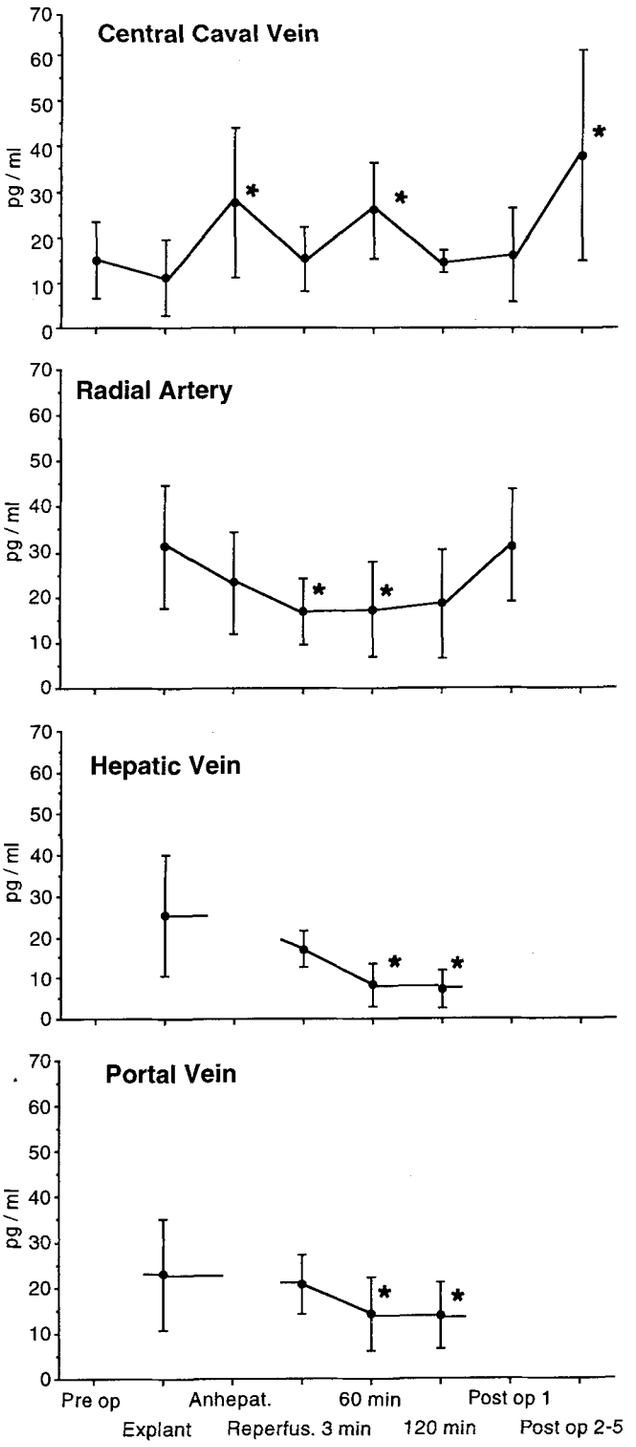

Fig. 1 Synchronous intraoperative big-ET plasma kinetics in different vessels during orthotopic liver transplantation (pg/ml) (* $P < 0.05$)

postoperatively after proof of good diuresis, blood levels 250–400 mg/dl by HPLC) and FK 506 (initial daily dose 0.15 mg/kg i. v.). Data are given as means ± SD in pg/ml (conversion to fmol: 1 fmol = 4.28 pg big-ET). Results in each group of values were compared using the Mann-Whitney *U* test; *P*-values < 0.05 were considered statistically significant.

Results

Big-ET plasma levels ranged from 0.8 to 98.0 pg/ml. Concentrations of big-ET at specific peri-, post- and intraoperative time points in the 12 selected patients are shown in Table 1 for all vascular compartments analysed. Stable intraoperative conditions were demonstrated by normotensive arterial blood pressure values and normal retention values. The postoperative increase in transaminases was moderate. Figure 1 separately illustrates the plasma kinetics of big-ET levels during OLT for each vessel separately. Differences in big-ET kinetics occurred in the various vascular compartments. In the central caval vein, highest concentrations were found during the anhepatic period and 60 min after reperfusion. A decrease extending to the first postoperative day was followed by an increase to a mean of 38 pg/ml during days 2–5. A temporary decrease was also noted immediately after reperfusion. During explantation of the liver, big-ET levels in the radial artery and hepatic and portal veins were significantly higher than caval levels. During the anhepatic period, big-ET levels in the radial artery and caval vein did not differ significantly. Highest levels in the hepatic and portal veins were detected at the end of the explantation period and after reperfusion, followed by a decrease in both vessels. A different pattern was found in the radial artery. Early postoperative levels were higher in the radial artery than in the caval vein.

Mean big-ET levels in the late postoperative course of all 28 patients were significantly ($P < 0.05$) elevated during stable graft function (30.0 ± 13.9 pg/ml, $n = 19$) compared with preoperative values (14.9 ± 8.3 pg/ml, $n = 28$). Big-ET plasma concentrations were significantly ($P < 0.05$) increased during episodes of infection (62.5 ± 26.5 pg/ml, $n = 10$) and acute graft rejection (39.9 ± 29.6 pg/ml, $n = 10$) compared with preoperative values and the stable graft situation.

Discussion

Various hepatic disorders are associated with increased systemic ET levels [1, 11, 15, 16, 27]. The following concentrations in cirrhotic liver disease have been found: 1.6 ± 0.2 pg/ml [11], 6.4 ± 1.8 pg/ml [1], 13 ± 2 pg/ml [15], 22.1 ± 4.7 [16]. Mean levels of 30.3 ± 8.5 pg/ml have been found in hepatocellular carcinoma [16] and of 36 ± 5 pg/ml in hepatorenal syndrome [15]. Inconsistency in the levels of ET found in liver cirrhosis could reflect a variable degree of hepatorenal impairment and amoung of sodium intake [1]. Big-ET levels have not yet been reported in a comparable series. Lerman et al. first reported elevated ET plasma levels (mean 4 pg/ml) during the first week after OLT [11]. Textor et al. found increased ET concentrations after OLT (mean 12.4 ± 2.7 pg/ml), which rose during the first week and fell back to baseline (1.5 ± 0.3 pg/ml) after 3 weeks [27]. In the current study, big-ET levels were markedly elevated after OLT. As found by Textor et al. for ET, a significant increase was not noted earlier than 24 h postoperatively big-ET has previously been reported to circulate in about equimolar concentrations to ET [5, 30, 31]. We found higher big-ET plasma levels after OLT, as reported for ET. The discrepancy could be derived from the instability and shorter half-life of ET during circulation or in frozen samples [22]. It may also point to a limited ET converting enzyme activity in states of reduced liver function [26] or may

Table 1 Big-ET plasma concentrations (pg/ml, means ± SD) before, during and soon after uncomplicated OLT in 12 selected patients. Stable intraoperative conditions were demonstrated by mean systolic/diastolic blood pressure values (mmHg), retention values (mg/100 ml) and a moderate increase in postoperative transaminase concentrations (SGOT, U/ml) (*n.d.* no investigation done)

	Caval vein	Radial artery	Hepatic vein	Portal vein	BP	Urea	Creatinue	SGOT
Preoperative	14.9 ± 8.3	n.d.	n.d.	n.d	118/66	43.6	1.0	169
Explantation	8.7 ± 8.5 ◇	31.3 ± 13.5 **	25.3 ± 14.7 **	20.4 ± 13.7 **	126/73	32.0	0.9	
Anhepatic phase	21.5 ± 18.6 ◇	23.2 ± 11.2	n.d.	n.d	116/65	34	0.8	
Reperfusion 3 min	11.8 ± 8.9	16.7 ± 7.3◇	17.0 ± 4.4 *◇	20.5 ± 6.5 **	120/60	29.5	1.0	n.d.
Reperfusion 60 min	20.2 ± 14.5 ◇	17.2 ± 10.5 ◇	8.2 ± 5.3 *◇	14.1 ± 7.9 ◇	115/62	31.7	1.1	
Reperfusion 120 min	11.5 ± 6.6	18.5 ± 11.9	8.1 ± 5.1 ◇	13.7 ± 7.3 ◇	118/66	29.1	1.1	
Postoperative day 1	12.5 ± 11.2	31.4 ± 12.3 **	n.d.	n.d.	135/83	39	1.0	850
Postoperative days 2–5	38.0 ± 23.0 ◇	n.d.	n.d.	n.d.	140/80	66.5	0.9	788

* $P < 0.05$; ** $P < 0.025$ vs caval values obtained simultaneously; ◇ $P < 0.05$

indicate direct big-ET release following by endothelial cell damage, since only very small amounts of big-ET have been detected in the culture supernatants of vital porcine aortic endothelial cells [5, 22, 30, 31].

Different mechanisms may contribute to ET/big-ET release during and after OLT. Increased ET/big-ET concentrations could reflect the insult to the recipient's entire vascular system after OLT [27]. Factors such as hypoxia, hypercapnia, acidosis, oxidant injury, hypovolaemia or fluctuations in hepatorenal function with secondary coagulopathy may be involved in endothelial stimulation [2, 18, 30, 31]. Thrombin [21], sudden changes in circulatory parameters or sheer vascular stress have been shown to enhance ET release [7, 8, 13]. Systemic ET levels could reflect a homeostatic response characterized by vasoconstriction plus sodium retention during hypotensive phases [7], such as the anhepatic period during OLT, or general vasodilation present in cirrhotic subjects. On the other hand, ET plasma concentrations of about 100 pg/ml far higher than detected in the current study after OLT, are the lowest levels that have been reported to induce systemic vasoconstriction [3, 30]. As pressor effects of ET have been found to be particularly potent in moderate hypoxia [12] even lower levels could have an impact in specific situations. ET levels measured by RIA may be lower than in reality as a decay of ET immunoreactivity has been reported after storage of frozen samples. Big-ET seems to be less affected by degradation [22].

Disturbances of splanchnic circulation may also increase ET levels by direct endothelial stimulation or by the impact of bacterial endotoxins on liver RES cells [18]. Sinusoidal endothelial liver cells produce ET in vitro [20], which can induce contraction of sinusoids by Ito cells. Elevation in portal pressure and a reduction in transhepatic flow has been shown in isolated perfused rat livers and in vivo after ET injections, suggesting a role of ETs in the regulation of hepatic microcirculation [9, 20, 28]. The highest levels in the portal and hepatic veins were found during explantation and reperfusion in our study. This also suggests ET generation in the liver and splanchnic circulation.

Injuries to the endothelial surface of the liver graft from immunological factors, endothelitis, preservation damage or secondary disturbances of hepatic microcirculation may also enhance ET plasma concentrations after OLT. As ET/big-ET seem not to be stored in endothelial cells [5, 13, 30], this hypothesis is controversial. Nevertheless, the recent detection of significant ET concentrations in the effluent of liver grafts after cold preservation suggests at least some ET accumulation in vascular spaces during harvesting and cold storage [25]. The perioperative detection of plasma big-ET, which is primarily an intracellular presecretion intermediate with (controversial) weak biological activity, further suggests passive ET release caused by endothelial membrane damage. Immunological activation of ET generation following allograft rejection and endothelitis is an attractive hypothesis. We detected increased non-specific elevations in big-ET levels during rejection episodes. Levels during severe systemic infections were also markedly elevated. As ET/big-ET levels have been shown to rise early during OLT or very early postoperatively, immunological factors appear to be of minor relevance at this early stage of antigen confrontation. Cultured endothelial cells release ET when challenged with CsA. Recent data show a correlation of a CsA-induced decrease in GFR, due to mesangial cell contraction [23], with increased urinary ET excretion but unchanged plasma ET levels. This suggests CsA-induced ET generation in the kidneys, possibly involved in CsA toxicity [17]. Because CsA was not given during OLT in our study, systemic intraoperative big-ET generation must have been induced by other stimuli. No postoperative correlation of ET values with CsA levels has yet been reported after OLT [11, 27].

ET-1 appears to be cleared rapidly from the circulation in vivo. The plasma half-life has been reported to range from 40 s to 7 min [24]. A major clearing site for ET is the lungs. Two-thirds of injected ET is removed by a single passage through the pulmonary circulation. The kidneys, liver, heart and spleen can take over clearance functions when pulmonary binding capacity is saturated [24]. The high arterial big-ET levels found in our study point to big-ET release in the peripheral arteries or the myocardial endothelium, as previously reported [14]. Rapid local clearance may to some extent explain the regional variation in plasma levels. Increased big-ET concentrations in both the caval vein and radial artery during the anhepatic period could have been caused by a reduction in clearance capacity [24]. Data on big-ET clearance and biological activity are limited. Processing of big-ET to ET is essential for full expression of vasoconstrictor activity [5, 22, 30, 31].

Sustained immunological endothelial stimulation could be involved in the generation of big-ET after OLT. The heterogeneity of the kinetics found in the different vascular sections during OLT suggests that the endothelial peptides act predominantly as local mediators released primarily abluminally in a paracrine way. Levels detected in the circulation may only be a fraction that has escaped transfer or binding to receptors [22]. Since ET-antagonizing effects of calcium channel blockers are

controversial [29], investigations focusing on blockage of ET receptors by the use of specific ET-receptor antagonists [6] could improve our understanding of the pathophysiological function of the ET peptides and allow better control of cardiovascular and renal dysfunction after OLT.

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