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Brief communication

Perioperative kinetics of the nitric oxide derivatives nitrite/nitrate during orthotopic liver transplantation

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Abstract

Nitric oxide (NO) is an important mediator in ischemia–reperfusion injury during human orthotopic liver transplantation (OLT). The perioperative kinetics of nitrite/nitrate plasma levels in 25 patients undergoing uncomplicated OLT were studied. A uniform pattern with significant increases of nitrite/nitrate levels immediately after reperfusion was seen in all patients, followed by a decrease to pretransplant levels within 24 h. Peak levels 30 min after reperfusion were correlated to the indocyanine green plasma disappearance rate (PDR_{ICG}), suggesting an association of early released NO with graft perfusion in OLT.

Keywords: OLT; NO; Nitrite/nitrate; PDR_{ICG}

Nitric oxide (NO) has been assigned an important role in ischemia–reperfusion (IR) injury during orthotopic liver transplantation (OLT). Most knowledge, however, is derived from animal models, where, depending on the experimental conditions, NO is able to decrease or enhance IR injury [1–3]. In both animal and human OLT a deficiency of the constitutively expressed NO synthase, which is primarily located in endothelial cells (eNOS), has been linked to tissue damage early in the reperfusion period, while increased activity of the inducible type of NO synthase (iNOS) in the later phase was associated with poor graft function [3–5]. The latter type of NOS is predominantly expressed by macrophages (e.g. Kuppfer cells) and neutrophils, and its induction by pro-inflammatory factors is linked to inflammatory processes and increased generation of highly reactive nitrogen species [5,6]. Thus, both the source as well as the timing of NO production during OLT might influence its role in IR, with early secreted, endothelial derived NO exerting protective functions by ameliorating disturbances in microvascular hepatic blood flow and by protecting liver cells from apoptotic and necrotic cell death [1–4].

To further investigate the role of NO in human OLT we examined the perioperative plasma levels of nitrite/nitrate, both stable end-products of NO, using high-performance liquid chromatography (HPLC). Levels were related to several clinical parameters including the indocyanine green plasma disappearance rate (PDR_{ICG}) as marker of perfusion and early function of the liver.

Methods

Study subjects

The study was carried out at the division of transplant surgery of the Medical University of Vienna, Austria. Twenty-five consecutive patients (11 females and 14 males) admitted for orthotopic liver transplantation were included in this study. Median age was 50 years (range, 36–64 years),

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indications for OLT were chronic active hepatitis C ($n=3$), chronic active hepatitis B ($n=1$), alcoholic cirrhosis ($n=12$), primary biliary cirrhosis ($n=3$), Wilson disease ($n=1$), hemangioma ($n=1$), and hepatocellular carcinoma ($n=4$). The median APACHE II score was 15 (range, 3–36). Liver transplantation was performed following the standard technique without veno-venous bypass but with cross-clamping of inferior vena cava. Either University of Wisconsin (UW) solution (6 grafts) or histidine–tryptophan–ketoglutarate (HTK) solution (19 grafts) was used for graft preservation. Prior to reperfusion, the liver grafts were flushed with cold human albumin (5%). Immunosuppression was started intraoperatively with 40 mg dexamethasone. Postoperative immunosuppression included dexamethasone (32 mg, day 1, tapered to 8 mg/day), antithymocyte-globulin (1 mg/kg/day, days 1–3) and cyclosporine (8 mg/kg/day divided into 2 doses, day 3).

Measurement of nitrite/nitrate levels

From all patients heparinized arterial blood samples were obtained through a radial artery catheter. Plasma was collected following centrifugation at 4 °C, aliquoted, and immediately frozen at −30 °C. Measurements were obtained at baseline immediately after induction of anesthesia, as well as 5 min, 30 min, 4 h, and 24 h after reperfusion of the graft, respectively. Total plasma levels of nitrite/nitrate were measured using high-performance liquid chromatography (HPLC) as described previously [7]. This sensitive method has been shown not to be influenced by the presence of biogenic amines. Values are expressed as μmol/L with a lower detection limit of 0.1 μmol/L. In 15 of 25 patients the PDR_{ICG} was studied for the assessment of liver perfusion and transplant function 20–40 min after finishing hepatic arterial anastomosis and subsequently on a daily basis (data not shown). Briefly, a 3-Fr fiberoptic catheter was advanced via the right or left femoral artery into the descending aorta (Pulsiocath, Pulsion, Munich, Germany). ICG (0.3 mg/kg body weight) was mixed in 10 mL of ice-cold 5% dextrose and was injected into the right atrium via a central venous catheter. Dilution curves for the dye were recorded in the descending aorta with the fiberoptic catheter. Mean transit times as well as exponential decay time for the dye indicator over a period of 240 s were determined with the COLD system (Pulsion Medical Systems, Munich, Germany). Results were used to calculate the plasma disappearance rate of ICG. Ethical clearance was given by the ethics committee of the University of Vienna.

Statistical analysis

Statistical analysis was performed using a standard statistical package (SPSS 11.5 for Windows; SPSS Inc., Chicago, IL). Nitrite/nitrate levels are expressed as mean values ± standard deviations. The Mann–Whitney *U*-test was applied for group differences (different preservation solutions). The Wilcoxon signed rank test was used to compare dependent variables within groups (nitrite/nitrate levels at different time points). Bivariate correlations were done by computing Spearman's correlation coefficient. A $p<0.05$ was considered significant.

Results

Among 25 consecutive enrolled patients who had undergone uneventful OLT, the median cold ischemia time was 7.5 h (range, 2–14 h). The median ICU length of stay was 5 days (range, 3–36 days). No episode of hyperacute graft rejection was observed among the study population, all graft recipients survived 28 days of follow up.

A nearly uniform pattern of the kinetics of nitrite/nitrate was observed during the perioperative period in our patients (Fig. 1). Nitrite/nitrate values were elevated after 5 min and increased further to maximum levels 30 min after reperfusion of the graft in all recipients. Four hours after reperfusion plasma levels were still elevated but then decreased to baseline (pretransplant) levels at 24 h.

Peak levels of nitrite/nitrate were strongly correlated to PDR_{ICG} suggesting a beneficial role of early high production of NO (Fig. 2). However, no correlations between nitrite/nitrate levels and other parameters such as age, Pugh-Child and APACHE II scores, cold ischemia time, creatinine-clearance, and the length of ICU stay were seen. Results were not influenced by the type of preservation solution used.

Discussion

This preliminary investigation shows that NO is released very early after reperfusion and returns to baseline (pretransplant) levels within 24 h in uncomplicated human OLT. Peak levels of nitrite/nitrate, both stable end-products of NO, were related to high PDR_{ICG} suggesting an

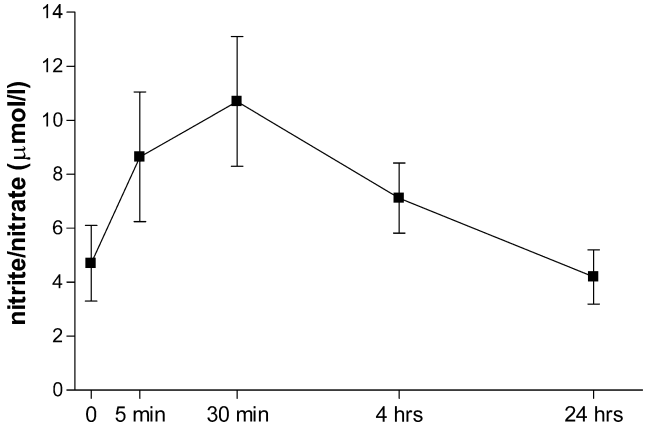

Fig. 1. Perioperative kinetics of nitrite/nitrate plasma levels as measured by high-performance liquid chromatography in 25 patients undergoing OLT. Values are expressed as mean ± standard deviation. A significant increase of nitrite/nitrate levels with a decline to pretransplant levels within 24 h was noted ($p<0.001$, as calculated by the Wilcoxon signed rank test). Nitrite/nitrate levels of healthy controls (data not shown) were always below 1.5 μmol/L.