Nitric Oxide 20 (2009) 61–67

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Brief Communication

The L-arginine/NO pathway in end-stage liver disease and during orthotopic liver and kidney transplantation: Biological and analytical ramifications

Thomas Becker [a], Iris Mevius [b], Dorottya K. de Vries [c], Alexander F.M. Schaapherder [c], Andreas Meyer zu Vilsendorf [a], Jürgen Klempnauer [a], Jürgen C. Frölich [b], Dimitrios Tsikas [b,*]

[a] Department of Visceral and Transplant Surgery, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany
[b] Institute of Clinical Pharmacology, Hannover Medical School, Carl-Neuberg-Strasse 1, D-30625 Hannover, Germany
[c] Department of Surgery, K6-R, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 21 March 2008
Revised 8 August 2008
Available online 10 October 2008

Keywords:
End-stage liver disease
Liver transplantation
Kidney transplantation
ADMA
DDAH
Proteolysis

ABSTRACT

The L-arginine/nitric oxide (L-Arg/NO) pathway is altered in liver and kidney diseases. However, the status of the L-Arg/NO pathway during and after orthotopic transplantation is insufficiently investigated and findings are uncertain because of analytical shortcomings. Also, most human studies have focused on individual members of the L-Arg/NO pathway such as nitrate or asymmetric dimethylarginine (ADMA). In the present article we report on a pilot study investigating extensively the status of the L-Arg/NO pathway before and during orthotopic liver transplantation (OLT). By using fully validated, highly sensitive and specific GC–MS and GC–MS/MS methods nitrite, nitrate, ADMA and its hydrolysis product dimethylamine (DMA), L-arginine and L-ornithine were measured in blood and urine. Our study gives strong evidence of the exceptional importance of hepatic dimethylarginine dimethylaminohydrolase (DDAH) activity for the elimination of systemic ADMA. In end-stage liver disease the synthesis of NO and ADMA as well as the DDAH activity are elevated. However, increase in DDAH activity is insufficient to efficiently eliminate overproduced ADMA. The transplanted liver graft is capable of clearing ADMA in a rapid and sufficient manner. In contrast to studies from other groups, our study shows that in OLT as well as in living donor kidney transplantation, the second study reported here, reperfusion of the graft does not cause drastic alterations to the L-Arg/NO pathway with regard to NO synthesis. In the OLT study the concentration of circulating L-arginine fell temporally dramatically, while L-ornithine levels increased diametrically, most likely due to elevation of arginase activity. However, the relatively long-lasting decrease in plasmatic L-arginine in OLT seems not to have affected NO synthesis after reperfusion. Our OLT study suggests that liver reperfusion is associated with greatly elevated activity of proteolytic and hydrolytic enzymes including DDAH and arginase. Suppression of proteolytic and hydrolytic activity in transplantation could be a useful measure to improve outcome and remains to be investigated in further studies on larger patient collectives. The importance of analytical chemistry in this area of research is also discussed in this article.

There is accumulating evidence that the L-arginine/nitric oxide (L-Arg/NO) pathway is altered in liver and kidney diseases [1]. Elevated circulating and excretory levels of nitrite and nitrate were found in patients with chronic liver disease and cirrhosis [2]. Serum nitrite + nitrate levels were found to correlate with disease stage and parameters of hyperdynamic circulation most likely induced by portal hypertension [2]. Elevated asymmetric dimethylarginine (ADMA) plasma concentrations in patients with chronic hepatic failure have been reported by Siroen et al. [3]. In the patients with end-stage liver disease involved in the present study (see Results section) we also found elevated excretion rates of dimethylamine (DMA), the enzymatic hydrolysis product of ADMA [4]. These findings suggest that ADMA synthesis is also elevated in end-stage liver disease and that hepatic and renal elimination via the dimethylarginine dimethylaminohydrolase (DDAH) metabolic pathway is apparently insufficient to compensate for ADMA overproduction. The rat and human liver have been shown to play an important role in the metabolism of ADMA by taking up large amounts from the systemic circulation [3,5]. Accumulation of ADMA could be a risk factor of early graft failure [6]. High plasma ADMA concentration was identified as an independent risk factor for multiple organ failure in critically ill patients causing an enhanced intensive care unit mortality [7,8]. It is also noteworthy that the transplanted liver graft is capable of clearing ADMA [9]. Thus, although not all members of the L-Arg/NO pathway have been investigated in liver disease so far, this pathway seems to

* Corresponding author. Fax: + 49 511 532 2750.
E-mail address: tsikas.dimitros@mh-hannover.de (D. Tsikas).

1089-8603/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.niox.2008.10.002

be up-regulated in hepatic failure. In particular, ADMA synthesis and elimination seem to be of key importance for liver, kidney and multiple organ failure.

The status of the L-Arg/NO pathway during and after OLT in humans on the basis of measurement in the circulation and in the urine of wide a spectrum of relevant biochemical parameters with indicator function is insufficiently investigated. In our opinion, these parameters should include nitrite and nitrate as indicators of NO synthase (NOS) activity, ADMA and DMA as measures for protein methylarginine transferase (PRMT) and DDAH activity, and L-arginine/L-ornithine for assessing arginase activity. Thus far, most human studies in this context focused on individual members of the L-Arg/NO pathway such as ADMA [9] or nitrite and nitrate [10].

Siroen and colleagues showed that ADMA plasma concentration decreased significantly from the pre-operative day to the first post-operative day and that ADMA concentration fell slowly to normal levels 1 year after transplantation [9]. In the study by Siroen et al. [9], plasmatic ADMA was determined by a highly specific and accurate HPLC method (reviewed in Ref. [11]), and the ADMA concentration measured by this method in plasma samples of healthy and ill humans, including patients suffering from liver disease, is in full agreement with literature data (for discussion see Refs. [12,13]).

In the recent study by Winkler and colleagues [10], 25 patients underwent OLT, and nitrite and nitrate were determined in plasma from heparinized arterial blood samples by a previously reported HPLC method [14]. Nitrite and nitrate concentrations, which have been reported as "nitrite/nitrate", were found in that study to be always below 1.5 μM in healthy controls and about 4.8 μM in the patients at baseline [10]. The reported plasma nitrite/nitrate concentrations measured in plasma of healthy controls of <1.5 μM are extremely low and in contradiction to the vast majority of literature data which were obtained by using various methodologies including HPLC [15,16], the Griess assay [16,17], GC–MS [16,18] and CE [19]. Also, the baseline plasma level of only about 4.8 μM for nitrite/nitrate measured in the patients immediately after anaesthesia in the study by Winkler et al. [10] are about 10–20 times lower than those reported by other groups for liver disease patients (also reviewed in Ref. [20]). Thus, as ever [21], we may reasonably call in question the analytical reliability of the HPLC method [14] used in the study by Winkler et al. [10] and the results obtained in that study. In particular, there is reasonable doubt about the doubling of the baseline level of nitrite/nitrate 5 min after reperfusion of the graft [10].

Thus, in addition to being limited to only one or two members of the L-Arg/NO family, many studies on liver and kidney transplantation suffer from validity mainly because of severe analytical shortcomings. Here, we report on the results from investigations on the status of the L-Arg/NO pathway in a pilot study on end-stage liver disease during orthotopic liver transplantation (OLT) in humans. The status of the L-Arg/NO pathway in the OLT study was determined extensively by quantifying in plasma and urine the following representative circulating and excretory members of this family: nitrite, nitrate, ADMA, DMA, L-arginine, L-ornithine. For this purpose, we used previously reported, fully validated and multiply approved, highly sensitive and specific GC–MS and GC–MS/MS analytical methods. To assess whether the results found in OLT are liver-specific or whether they are generally related to ischemia/reperfusion (I/R) injury, we measured plasma nitrite and nitrate levels after reperfusion in a pilot human kidney transplantation study. In consideration of the contradictory results reported in the literature from transplantation studies, we discuss in this article the importance of the validity of analytical methods in clinical studies.

Materials and methods

Orthotopic liver transplantation study

Nine patients (7 males, mean age 45.7 ± 14.7 years, range 32–69; 2 females of 42 and 58 years of age) with end-stage liver disease and scheduled for liver transplantation were included in the study. The study protocol was approved by the local Ethics Committee and was performed according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all patients. Patient characteristics und diagnosis is summarized in Table 1. Hepatic disease included liver cirrhosis for hepatitis C ($n = 1$), autoimmune hepatitis ($n = 2$), alcoholic cirrhosis ($n = 2$), Budd–Chiari syndrome ($n = 2$) and cholestatic disease ($n = 2$). Five of these patients were in Child–Pugh stage B. One patient died from unknown reason as an outclinic patient after an uneventful post-operative course on day 47, and one patient died because of tumour recurrence 28 months after transplantation. All other patients are still alive with good graft function. All patients underwent full-size OLT in standard technique without veno-venous bypass between March 2002 and August 2002 in the Hannover Medical School. Patients and healthy volunteers did not follow a standardized low nitrite/nitrate diet in the present study.

Kidney transplantation study

Six eligible patients (2 males, mean age 41.2 ± 11.0 years) undergoing renal allograft transplantation with living donors between October 2004 and June 2005 in the Leiden University Medical Centre were recruited. The study protocol was approved by the local Ethics Committee, and informed consent was obtained preoperatively from each patient. Living donor kidney transplantation was selected as model for I/R injury because of the high level of homogeneity in cold ischemic period. Living donor kidney transplantations were performed according to local standardized protocol. The donors (4 males, mean age 41.0 ± 13.1 years) underwent open minimally invasive donor nephrectomy. Mean cold ischemic period was 177 ± 21 min. All grafts started urine production within half an hour of reperfusion. Post-operative course was uneventful in all patients. One year patient and graft survival was 100%.

Sampling protocols

In the OLT study, serial blood samples (8–10 ml) were taken from a peripheral vein or from central venous catheter by using disposable blood sampling tubes containing EDTA for anticoagulation from Sarstedt (Nümbrecht, Germany) at the following time points: pre-operatively, before anaesthesia, after skin incision, 1 min before unhepatic clamping time, 0, 5, 10, 20, 40, 60, 120 and 240 min after reperfusion; data obtained from pre-operative blood sampling were used as baseline levels. In addition, blood samples were drawn from the clamped recipient portal vein immediately before reperfusion and from the graft efflux through the infrahepatic vein by flushing the graft. Plasma was generated by immediate centrifugation (800*g*, 5 min, 2 °C), aliquoted appropriately for the single plasma parameters and stored at −80 °C until analysis. Urine samples were collected pre-operatively (U1) from spontaneous micturition, 40–60 min after reperfusion (U2), and 60–240 min after reperfusion (U3) by means of a urethral catheter. Urine samples were aliquoted in 40-ml portions and stored at −80 °C until analysis. Concentration of urinary analytes was corrected for creatinine, the concentration of which was determined by the picric method. Creatinine-corrected excretion rate was expressed as μmol of analyte per mmol of creatinine.

In the kidney transplantation study, arterial blood was sampled from the indwelling arterial line. At 0, 3, 5, 10, 20, 30 and 45 min