Influence of N-acetylcysteine on hepatic amino acid metabolism in patients undergoing orthotopic liver transplantation

J. Thies
Department of Transplantation and Hepatobiliary Surgery,
University of Mainz, Langenbeckstrasse 1, 55101 Mainz, Germany

Abstract Experimental treatment with the antioxidant and glutathione precursor N-acetylcysteine (NAC) has been performed in orthotopic liver transplantation (OLT) to reduce reperfusion injury. To investigate the effect of NAC on the hepatic and intestinal amino acid metabolism, intraoperative amino acid exchange rates were studied in liver transplant recipients with high dose NAC treatment \((n = 10)\) and in control patients \((n = 9)\). Treatment with NAC was found to cause a loss of amino acids and increased urea nitrogen release from the liver graft. The net balance of most amino acids was shifted to increased hepatic release or decreased hepatic uptake. The initial cumulative splanchnic release of all proteinogenic amino acids in the NAC treated group was significantly higher than in the control group. These findings are tentatively explained by an increased net protein catabolism in the liver. The increased hepatic urea and glutamine production rate of the NAC treated patients is expected to increase the energy and oxygen demand of the liver in this critical situation. Thus, NAC may have caused marked metabolic disturbances in the freshly implanted graft. The dosage of NAC should therefore be modified to avoid these disadvantages.

Keywords Antioxidants · Reperfusion · Substrate balances · Urea production · Transplantation · Cysteine

Abbreviations NAC N-acetylcysteine · BCAA Branched-chain amino acids · AA Aromatic amino acids · PG-AA Proteinogenic amino acids

Introduction High dose therapy with N-acetylcysteine (NAC) has become part of the standard treatment for patients with acute hepatic failure. Originally, the regimen was developed for hepatic failure due to paracetamol (acetaminophen) poisoning \([8, 15]\). NAC has become an important treatment strategy also in the pharmacotherapy of acute hepatic failure from other causes \([5]\). The pathological consequences of paracetamol poisoning and the striking therapeutic effects of NAC in this context exemplify the importance of the intracellular glutathione concentration and the availability of the glutathione precursor cysteine for the maintenance of liver function. It has also been hypothesised that cyst(e)ine is catabolized in the liver to sulphate and protons and may thereby con-
Contribute to the regulation of nitrogen disposal. Specifically proton generating processes are expected to downregulate urea formation in favour of glutamine biosynthesis [4]. We have shown that NAC is rapidly catabolized into inorganic sulphate by the splanchnic tissues, possibly leading to a generation of an excess of protons [18].

In organ transplantation, prevention of tissue damage from reactive oxygen intermediates (ischemia-reperfusion injury) gives a rationale for the use of NAC as a radical scavenger. Beyond the radical scavenging properties, less specific modes of action such as improvement in hemodynamics may contribute to its positive effects: NAC has vasorelaxant properties by formation of S-nitrosocysteine [16]. In animal transplantation models, NAC treatment improved sinusoidal hemodynamics [10, 11, 14]. Consequently, a clinical study has been conducted showing improved liver hemodynamics and synthesis function, as well as a decreased rate of primary graft failure in patients with high dose NAC application during liver transplantation compared with control liver transplant recipients [20].

In view of these hypotheses and preliminary findings, we now assessed the influence of intraoperative NAC treatment on the nitrogen metabolism of the freshly implanted liver grafts in the same clinical setting.

Patients and methods

With institutional approval, 20 consecutive patients receiving orthotopic liver transplantation were included in the study on NAC after informed consent. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Indications for transplantation included cirrhosis for alcohol abuse, chronic hepatitis, hemochromatosis, amyloidosis, Wilson’s disease, primary biliary cirrhosis and hepatocellular carcinoma. Age at time of transplantation was 54.0 ± 5.5 years in the NAC-group and 47.7 ± 15.0 years in controls (mean ± S.D.; difference n.s. [not significant]). Immunosuppressive therapy with prednisolone and either cyclosporine A or tacrolimus was initiated at reperfusion.

In an alternate fashion, the patients either received NAC dissolved in 5% glucose solution at 150 mg/kg of body weight during the following 4 hours, and 100 mg/kg during the following 16 hours, or received the same volume of 5% glucose without NAC to serve as controls. Livers for patients in the treatment group were rinsed with 1 liter Ringer’s solution containing 1 g NAC / liter immediately before implantation.

Sample collection and analysis

One hour after graft reperfusion, blood flow in the hepatic artery and the portal vein was measured by Doppler sonographic flow measurement [12]. At the same time, arterial blood was drawn from the arterial line, portal venous as well as a hepatic venous blood was collected by direct puncture of the respective vessels. Samples were also drawn from a central venous catheter 6 hours, 48 hours, 4 days and 8 days after surgery. Samples were collected in heparinized tubes and immediately cooled on ice; plasma was separated by centrifugation within one hour and frozen at −20°C.

After deproteinizing plasma samples with sulfosalicylic acid (50%, 1 : 15 vol/vol.) and centrifugation at 400 g for 10 min at + 4°C, amino acid concentrations were measured by high pressure liquid chromatography (Biotronik LC 3000 Amino Acid Analyzer, Durum Column, Eppendorf, Hamburg, Germany). Analysis was restricted to patients with moderately to well functioning liver grafts. Exclusion criteria were failure of the liver graft to clear phenylalanine to systemic concentrations < 100 μmol/l and tyrosine to systemic concentrations < 150 μmol/l [3, 6] by 6 hours postoperatively. One female patient had to be excluded from the control group due to high postoperative concentrations of phenylalanine (125.2 μmol/l) and tyrosine (413 μmol/l).

Calculations of amino acid balances and statistics

Individual hematocrit values at the time of flow measurement were used to calculate plasma flow from blood flow by multiplying by (1 – hematocrit). Mean hematocrit value was 0.28. The sum of hepatic arterial and portal venous plasma flows was taken for hepatic venous plasma flow.

Amino acid balances (μmol * min⁻¹) were calculated as the arteriovenous differences multiplied by plasma flow [13]. For net hepatic balance, hepatic arterial and portal flow were considered according to their actually measured ratio.

“Intestinal balance” refers to the portal-draining viscera (extrahepatic splanchnic tissues), “splanchnic balance” refers to the portal-draining viscera plus liver. Positive values denote a net uptake, negative values denote a net release of substrates.

All data in text, table, and figures are presented as mean ± SEM (standard error of the mean). The U-test by Mann, Whitney, and Wilcoxon (two-tailed) was used for statistical analysis. Significant differences between the two groups were mainly seen among the splanchnic exchange rates. Hepatic exchange rates are valuable for topical differentiation between liver and intestinal metabolism, but tend to be less precise since they depend on six different factors (concentration and flow in three different vessels).

Results

Systemic plasma concentrations of the total proteinogenic amino acids (PG-AA)

The systemic plasma concentrations of the total proteinogenic amino acids (PG-AA) of the two treatment groups were, on the average, not significantly different. The intraoperative arterial and early postoperative central venous plasma PG-AA levels were 2.87 ± 0.22 and 2.01 ± 0.13 mmol/l, respectively in the NAC-treated group and 2.48 ± 0.15 and 1.79 ± 0.10 mmol/l, respectively in the control group. The mean PG-AA concentrations of all 19 patients from both groups at days 2, 4 and 8 after transplantation were 2.03 ± 0.11, 2.88 ± 0.21 and 2.37 ± 0.09 mmol/l, respectively.