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

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This work is dedicated to our student Alexander Babylon who died after a tragic accident

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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-

tribute 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]). Immunosupressive 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 15 minutes after reperfusion, 50 mg/kg 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 vessel. 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 Analyser, 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.

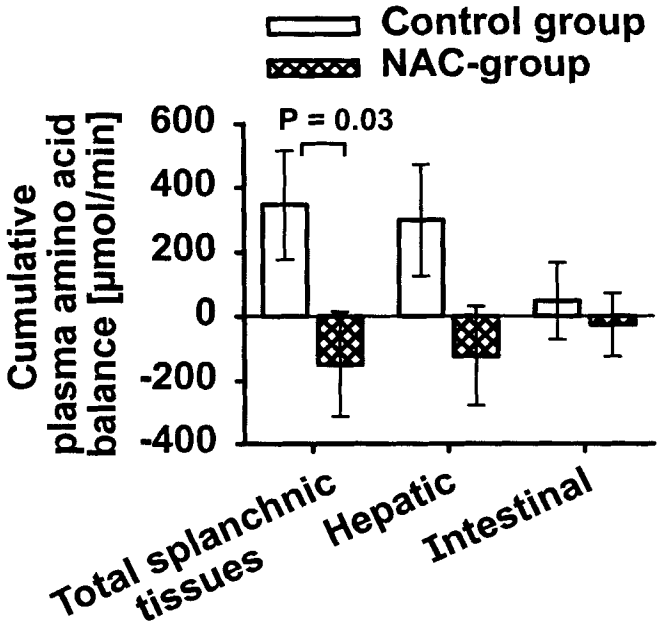

Fig. 1 Exchange rates of the cumulated proteinogenic amino acids across the total splanchnic tissues, the liver, and the extrahepatic splanchnic tissues. The data show the mean of the exchange rates ± S.E.M. of the NAC-treated ($n = 10$) and control patients ($n = 9$) one hour after reperfusion of the liver graft. Positive values indicate an uptake, negative values indicate the release of amino acids

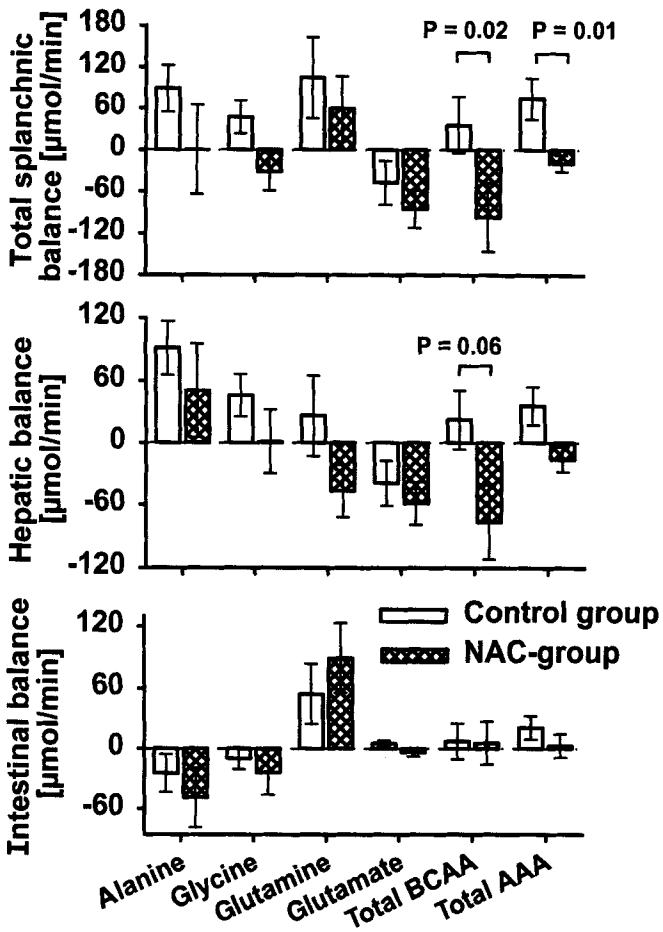

Fig. 2 Single amino acid exchange rates across the total splanchnic tissues, the liver and the extrahepatic splanchnic tissues. Balances of the indicated amino acids and cumulated branched chain amino acids and aromatic amino acids one hour after reperfusion of the liver graft (mean ± S.E.M.)

Amino acid exchange rates

Cumulative balance of the PG-AA

The analysis of the amino acid exchange across the total splanchnic tissues revealed, on the average, a net release of PG-AA in the case of the NAC-treated group and a substantial net uptake in the control group. This difference was statistically significant (Fig. 1). The more detailed analysis showed that the net release of the NAC group and the net uptake in the control group was essentially contributed by the liver, whereas the intestinal (i.e. the nonhepatic splanchnic) tissues showed in either case only a minute uptake or release, respectively.

Exchange rates of selected amino acids across the liver and nonhepatic splanchnic tissues

The exchange rates of several nonessential amino acids, branched chain amino acids (BCAA) and aromatic amino acids (AAA) across the total splanchnic tissues one hour after reperfusion showed again that the NAC-treated group had generally either a lesser uptake or a more profound release of amino acids than the control groups. This difference was statistically significant in the case of the BCAA ($P = 0.02$) and AAA ($P = 0.01$). The hepatic balance was in most cases similar to the balance of the total splanchnic tissues, indicating that the differences were mostly contributed by metabolic differences in the liver. The intestinal tissue showed, expectedly, a net uptake of glutamine and a release of alanine in the case of both treatment groups but little uptake or release of other amino acids (Fig. 2).

Urea production

One hour after reperfusion the NAC-treated group also showed a markedly enhanced rate of urea production. This difference was statistically significant in the case of the total splanchnic tissues that was, expectedly, due to an increased rate of urea production in the liver (Fig. 3). As a consequence, the NAC-treated group

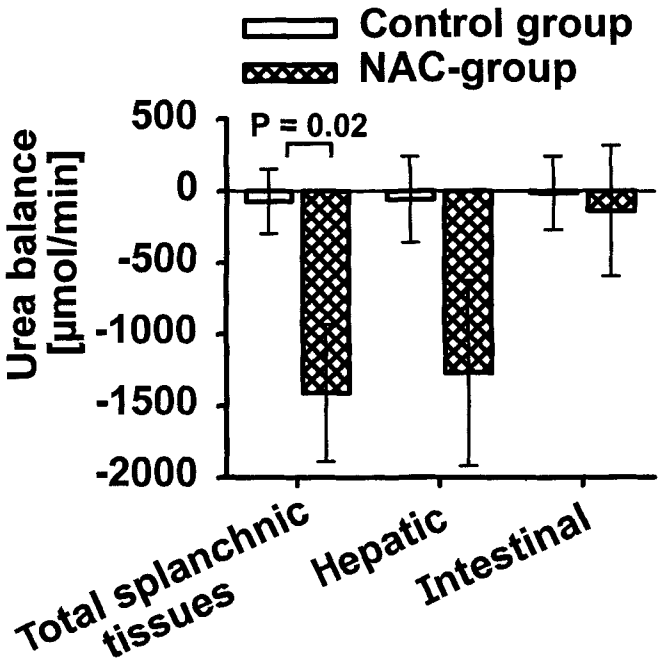

Fig. 3 Urea production. Urea balances of the total splanchnic tissues, the liver and the extrahepatic splanchnic tissues one hour after reperfusion of the liver graft (mean ± S.E.M.)

showed, on the average, a 50% higher urea concentration 6 hours postoperatively in comparison to the control group (data not shown).

Discussion

Our study revealed that high dose NAC treatment has a profound effect on the splanchnic amino acid balance in patients undergoing orthotopic liver transplantation. The conspicuous shift from a cumulated net uptake of PG-AA to a net release and especially the increased hepatic release of urea, glutamine and BCAA strongly suggest that NAC induces a net protein catabolism in the liver. The massive loss of urea nitrogen of approximately 3.6 mmol/min in the NAC-treated patients one hour after reperfusion exceeds by far the amount of nitrogen that was given to these patients in the form of NAC. One hour after reperfusion, these patients had received approximately one mmol NAC/kg body weight. Moreover, the net release of sulphur across the splanchnic tissues was higher in the NAC-treated group than in the control group by approximately 130 µmol/min only [18]. The mechanisms of these NAC-mediated changes in the hepatic nitrogen balance are not known. Tentatively, we propose that NAC may induce an increased biosynthesis of glutathione in the liver. This is expected to drain the intracellular energy stores. The drainage of cellular ATP may be expected to be further increased by two other energy consuming processes, i.e. the production of glutamine and urea. The resulting oxygen demand may possibly exceed the hepatic oxygen supply in the postreperfusion period and may therefore compromise the hepatic net protein synthesis. It was previously suggested that the net uptake of amino acids by the liver, i.e. the central plasma amino acid clearance, may be considered as a measure of the functional integrity of the liver [1].

The increase in net nitrogen loss from the liver of NAC-treated transplant patients was surprising in view of the hepatoprotective effects of NAC in a recent series of liver transplantations [19]. However, two earlier studies with NAC in OLT had failed to show improved outcome [2, 17]. Adverse effects of NAC had previously been found in experimental settings, where the integrity of the intestinal mucous layer was compromised by high doses of this drug [7, 9]. We do not know whether similar mechanisms apply here.

Our study only reflects the intraoperative and immediate postoperative situation giving some insight into early NAC effects. With the small number of patients studied here it was not intended to show differences in clinical outcome between the groups. Additional studies including more patients may be needed to better understand the mechanisms involved and to identify the optimal doses of NAC that provide the best hepatoprotective effects without the risk of unwanted effects, namely net hepatic protein catabolism.

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