N-Acetylcysteine failed to improve early microcirculatory alterations of the rat liver after transplantation

Abstract The application of radical scavengers reduces reperfusion injury of liver grafts despite the natural occurrence of cellular defense mechanisms enabling the cell to tolerate moderate oxidant stress without further cell damage. The glutathione peroxidase mechanism of the liver serves to reduce hydroxyl radical-induced lipid peroxidation by releasing reduced glutathione from intracellular stores. There is evidence that the application of cysteine-providing aminoacids for glutathione synthesis could maintain or even increase liver glutathione. Therefore, the purpose of this study was to evaluate the effect of N-acetylcysteine (NAC) on oxidative stress-induced reperfusion injury after liver transplantation. This was done by applying intravital microscopy. Livers from female Sprague-Dawley rats weighing 220–260 g were stored for 20 h in University of Wisconsin (UW) solution and transplanted orthotopically using the cuff technique. Donors were given 150 mg/kg body weight NAC i. v. or placebo at the beginning of the recipient operations, 1 min prior to reperfusion, and 60 min after surgery. Ninety minutes after transplantation, intravital microscopy was applied and five liver lobules were recorded for 30 s after injection of acridine orange, a fluorescent leukocyte marker. Sinusoidal perfusion, sinusoidal width, and leukocyte adhesion, as well as reduced and oxidized glutathione, were determined in all livers. Neither microcirculatory disturbance nor leukocyte adhesion was less, nor was the liver glutathione in the recipient groups pretreated or treated with NAC greater than that in rats receiving the placebo. Moreover, liver glutathione was significantly decreased in livers from donors pretreated with NAC. In conclusion, the application of NAC as a pretreatment for donors and as treatment for recipients, respectively, failed to reduce early microvascular failure after liver transplantation.

Key words Liver transplantation, rat, N-acetylcysteine - N-acetylcysteine, rat, liver transplantation - Preservation, rat liver, N-acetylcysteine - Microcirculation, N-acetylcysteine, liver preservation
**Introduction**

It is generally known that oxygen free radicals (OFR) are associated with reperfusion injury in transplanted livers [7]. During cold ischemia, adenosine triphosphate (ATP) is metabolized to adenosine, inosine, and hypoxanthine, and xanthine dehydrogenase is converted to xanthine oxidase [26]. In the presence of molecular oxygen during reperfusion, reactive superoxide anions and hydrogen peroxides are generated [9]. The subsequent formation of hydroxyl radicals requires the catalytic effect of the Haber Weiß reaction in the presence of iron [9, 20]. The highly damaging hydroxyl radical reacts with unsaturated membrane-bound lipids, causing severe endothelial cell damage by lipid peroxidation [10, 35].

Nonparenchymal cells of the hepatic sinusoids, especially resident macrophages (Kupffer cells) are known to be another important source of OFR [13]. Gut-derived endotoxin leads to the calcium-dependent activation of Kupffer cells [33, 34], releasing interleukin-1 (IL-1), tumor necrosis factor (TNF) and OFR [37]. We demonstrated previously that OFR may cause the adhesion of leukocytes to the endothelium [23]. The expression of adhesion molecules, mainly selectins and integrins, is mediated in part by OFR [27], leading to the accumulation of leukocytes in reperfused livers [19, 23]. Furthermore, it is known that increased leukocyte adhesion is associated with microcirculation disturbance and graft dysfunction.

On the one hand, a variety of radical scavengers have been introduced in experimental liver transplantation in the last decade [6, 23, 32], demonstrating that new strategies of organ procurement and postoperative management have succeeded in reducing reperfusion injury of transplanted grafts. The influence of preservation solutions containing different radical scavengers on microcirculation during reperfusion has recently been investigated by our group [21, 38]. On the other hand, different protective mechanisms, e.g., superoxide dismutase, catalase, and glutathione peroxidase, are naturally available in each cell to minimize the harmful effects of OFR. Glutathione (GSH) is one important component of the cells that is responsible for the structural and functional integrity of cell membranes. The major intracellular thiol [25] is synthesized from glycin, glutamate, and the thiol providing aminoacid cystein. During reperfusion, reduced GSH could act as a relevant radical scavenger [25], released from parenchymal liver cells [11]. It has been reported that an intact GSH peroxidase system during reperfusion can attenuate oxidant stress without further cell damage [15]. Otherwise, depletion of liver GSH by GSH-reducing agents is directly related to increased lipid peroxidation in the liver [5].

Since GSH was found to be reduced after cold ischemia [36], GSH is added to University of Wisconsin (UW) solution to maintain the endogenous defense mechanisms [3]. While cell membranes, in general, are impermeable to GSH, it is said that GSH is metabolized, providing amino acid cysteine, which is the rate-limiting substance for the intracellular synthesis of GSH [1]. Yu et al. have demonstrated that GSH, apart from other ingredients, is an essential component of UW solution, necessary for improved survival after rat liver transplantation [40].

Apart from the improvement in cellular integrity during cold ischemia, there is evidence that the GSH level in the liver could be maintained or even increased to some extent by the application of precursors of GSH [1, 14, 30]. In this respect, it has been shown that lipid peroxidation and the release of liver enzymes are mitigated by pretreatment of rats prior to and following liver transplantation with γ-glutamylcysteine ethyl ester [18]. The application of GSH precursors prior to ischemia maintained the integrity of liver function following reperfusion [8, 12, 18].

The purpose of this study was, therefore, to determine the effect of N-acetylcysteine (NAC) pretreatment of the donor on liver GSH and on microcirculation after orthotopic liver transplantation in the rat. Apart from the possible improvement in the endogenous GSH peroxidase mechanism, substances related to GSH, such as NAC, possibly act as powerful scavengers of hydroxyl radicals [2]. Therefore, in additional experiments NAC was given to recipients to investigate the reducing capacity of NAC during early reperfusion.

**Materials and methods**

**Animals**

Female Sprague-Dawley rats weighing 220–260 g (Hannoversche Tierversuchsanstalt, Hannover, Germany) were used as donors and recipients. The animals had free access to water and standard rat chow until the beginning of the experiments. Harvesting was performed between 3 and 4 p.m., followed by transplantation at 11 a.m. and 12 noon of the following day. General anesthesia with ether was given during all surgical procedures. Ninety minutes after transplantation, the rats were reanesthetized with pentobarbital i.v. (20–50 mg/kg body weight, Narkoren, Rhone Merieux, Laubheim, Germany). At the end of the experiments the rats were sacrificed via an overdose of pentobarbital, and the livers were resected via an overdose of pentobarbital, and the livers were removed to determine the GSH. The experiments were conducted after permission had been given by the local ethics committee and were done in accordance with the NIH guidelines for the use of laboratory animals.

**Transplantation procedure**

Twenty-four livers were transplanted orthotopically after cold storage (0–4°C) for 20 h in UW solution (Via Span, DuPont Pharma, Germany/NBPI, The Netherlands) using the cuff technique developed by Kamada and Calne and by Zimmermann and colleagues [17, 41]. Before harvesting the organ, an intraluminal stent was in-