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Extended preservation and effect of nitric oxide production in liver transplantation

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Abstract Liver transplantation (Ltx) has become a routine procedure for patients with end-stage liver disease. Despite ongoing progress on short- and long-term graft survival, primary dysfunction (PDF) remains a major problem. PDF is significantly associated with the duration of cold ischemia- and, possibly, with reperfusion-related injury. Nitric oxide (NO) has many physiological functions and plays an important role in modulating tissue injury. However, the mechanism of NO action in ischemia/reperfusion injury after Ltx is thus far unknown. In this study we investigated the role of inducable NO synthase (iNOS) in the liver after preservation with UW solution using the orthotopic Ltx model in the rat. Male Brown Norway rats were used for the Ltx procedure. After donor hepatectomy, livers were stored on ice-cold UW solution for 24 or 40 h and subsequently transplanted. A control group consisted of rats with Ltx after less than 1 h storage. Post-transplant blood samples were taken at 48 h to determine standard parameters for liver injury (aspartate transaminase, lactate dehydrogenase). Liver biopsies were obtained for detection of expression of iNOS (western blot) 24 and 48 h post-transplant. We observed that a preservation time of 24 h in UW solution presents no problem for graft survival after Ltx in rats with some brain function and in healthy animals. After 40 h preservation, liver damage is obvious and graft survival reduced, indicating the limits of cold storage may be within reach. With longer preservation times, more NOs was detected in liver tissue. This finding suggests that NO has a role in ischemia/reperfusion-related injury. Current intervention with NOS inhibitors will reveal whether NO has a negative or a positive effect on graft survival after Ltx.

Key words Liver transplantation · Ischemia and reperfusion injury · Nitric oxide

Introduction

Primary dysfunction of the liver due to primary non-function or initial poor function and ischemic biliary complications remain a major problem in Ltx. All three complications are associated with the duration of cold ischemia and, possibly, with reperfusion-related injury [3]. Cold ischemia- and reperfusion-related injury are multifactorial and involve a number of mediators. Nitric oxide (NO) plays a important role in both physiological and pathological processes and could be a modulating factor in ischemia and reperfusion injury. The relationship between cold ischemia- and reperfusion-related injury and NO is thus far unknown.

We have previously shown a significant rise of nitrite/nitrate (NO_x) levels, the stable end product of NO, in the flush-out solution after different times of preservation, suggesting NO synthase (NOS) activity during

cold storage (CS) with UW solution. Increased levels, however, were not related to preservation time and not influenced by NOS inhibitors. This could have been caused by the limitations of the in vitro model used.

In this study we have evaluated the effect of cold storage time on NO synthesis and the hepatic expression of inducible NOS (iNOS) and assessed a potential relationship with posttransplant function and survival after orthotopic Ltx in the rat.

Materials and methods

Male Brown Norway rats (body weight 250–300 g) were used for the Ltx procedure. After donor hepatectomy, livers were stored in ice-cold UW solution for 24 h ($n = 7$) or 40 h ($n = 9$) and subsequently transplanted using the Kamada technique [1]. In the control group, Ltx was performed immediately after retrieval and flushing with UW solution ($n = 9$). NO_x levels were determined in the serum of the liver donor and in the recipient directly after reflow and 24 h and 48 h after the Ltx procedure. During the Ltx procedure, after CS but prior to reflow, flush-out solutions were collected for sampling of aspartate transaminase (AST) and lactate dehydrogenase (LDH) levels. Blood samples were taken to determine standard parameters for liver injury 48 h after transplantation. At 48 h after Ltx, the transplanted rats were killed and liver biopsies were obtained to detect iNOS expression. NO_x levels were determined by the Griess reaction after nitrate reduction [2]. AST and LDH levels were calculated using routine clinical chemistry. Expression of iNOS was analyzed by western blotting, using a specific polyclonal antibody against the C-terminal portion of rat iNOS.

Results

Survival of control animals (6/6) and after 24 h CS (7/7) was 100%. After 40 h, CS animal survival decreased to 45% (4/9) as shown in Table 1. In liver donors, NO_x levels were in a physiological range (16.3 ± 2.1 μM). After Ltx, up to 24 h, no significant increase in NO_x level was seen (31.2 ± 1.9 μM). After 48 h postLtx, NO_x levels in both CS groups increased with the longer duration of CS, as shown in Table 2 (control 35.4 ± 4.3, 24 h CS 52.7 ± 14.9, 40 h CS 68.3 ± 19.2 μM). The LDH level in the flush-out solution was significantly increased after 24 h and 40 h CS compared to control livers (1022.4 ± 22.2 and 1156.0 ± 42.1, respectively, vs 338.2 ± 15.4 U/l). No significant differences between groups with and without CS prior to Ltx were observed for AST levels. Posttransplant AST and LDH serum levels increased with the length of CS time, reflecting tissue injury (AST controls 231 ± 75.8, 24 h CS 474 ± 106.8, 40 h CS 1736 ± 116.8 U/l; LDH control 220 ± 56.0, 24 h CS 261 ± 48.2; 40 h CS 460.0 ± 108.9 U/l). This increase was statistically significant for AST levels ($P < 0.05$) and a trend was found for LDH levels ($P = 0.08$). No detectable iNOS expression in the liver biopsies of non-preserved controls was observed by western blot analysis. After 24 h, CS iNOS expression was moderately induced. After 40 h, CS iNOS induction was profound and significantly increased in all experimental animals, as shown in Table 1.

Table 1 Survival and inducible nitric oxide synthase *(iNOS)* expression after different times of cold storage *(CS)*

	Survival *(n)*	Survival (%)	iNOS expression
Control	6/6	100	0
24 h CS	7/7	100	+
40 h CS	4/9	45	+++

Table 2 Nitrite/nitrate levels (in μM) in serum after different times of cold storage *(CS)* prior to liver transplantation *(Ltx)*

	Donor	Recipient		
		0 h postLtx	24 h postLtx	48 h postLtx
Control	19.2 ± 4.9	19.8 ± 2.7	28.0 ± 1.6	35.4 ± 4.3
24 h CS	9.4 ± 2.0	13.4 ± 2.0	28.3 ± 3.1	52.7 ± 14.9
40 h CS	17.9 ± 3.2	24.2 ± 5.3	39.2 ± 2.4	68.3 ± 19.2

Discussion

Primary dysfunction remains a major problem in Ltx associated with significant morbidity, retransplantation rate, and mortality. NO as a mediator of a variety of physiological functions might also be involved in modulating ischemia and reperfusion tissue injury resulting in primary dysfunction. We have previously shown significantly raised NO_x levels in flush-out solution after different times of preservation, suggesting NOS activity during cold ischemia (0–4 °C). Increased levels, however, were not related to preservation time and not influenced by NOS inhibitors. This could have been caused by the limitations of the in vitro model used. In this study we have analyzed the effect of CS with UW solution on NO synthesis in Ltx in the rat. NO synthesis was assessed by expression of iNOS in liver tissue and NO_x levels in the serum. The potential relationship with posttransplant function and survival was evaluated. The experimental model used shows a reproducible degree of ischemia- and reperfusion-related injury. With prolonged CS times, an increase in tissue injury is seen, which is reflected in a decreased posttransplant function. Animal survival up to 24 h CS with UW solution is 100%. When CS is prolonged to 40 h, survival is significantly reduced by 50% and coincides with significantly elevated serum AST levels. A concomitant increase in NO_x, profound iNOS expression, and significant reduction in survival after 40 h CS might indicate NO involvement as a mediator for modulating ische-

mia- and reperfusion-related tissue injury. To distinguish between a positive or detrimental effect of NO, specific inhibitors of iNOS or endogenous NOS are necessary. Progressive loss of function and lower animal survival after CS and Ltx in the presence of an inhibitor of NO production indicates a protective effect of NO, while improvement after adding the inhibiting agent signifies a detrimental effect of NO in the rat Ltx model.

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References

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