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Assessment of hepatic graft injury by graft effluent in rodents: *N*-acetyl-*β*-glucosaminidase and type III procollagen peptide

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Abstract We studied the significance of *N*-acetyl-β-glucosaminidase (β-NAG) and type III procollagen peptide (P-III-P) in the effluent of rodent hepatic grafts. After total hepatectomy, the livers were preserved in chilled, lactated Ringer's solution and then divided into five groups ($n = 10$ each): group 1, 4 h preservation only; group 2, 4 h preservation and rewarming; group 3, 6 h preservation only; group 4, 6 h preservation and rewarming; and group 5, minimal preservation only. The β-NAG of groups 2 and 4 was significantly higher than that of groups 1 and 3 (0.98 ± 0.5 U/l vs 0.21 ± 0.12 U/l; $P < 0.01$ and 1.76 ± 0.67 U/l vs 0.38 ± 0.25 U/l, respectively; $P < 0.01$), while that of group 4 was significantly higher than that of group 2 (1.76 ± 0.67 U/l vs 0.98 ± 0.50 U/l; $P < 0.05$). The P-III-P of group 4 was significantly higher than that of group 2 (0.133 ± 0.008 U/ml vs 0.110 ± 0.015 U/ml; $P < 0.01$). We conclude that β-NAG is a novel parameter of parenchymal and nonparenchymal cells, while P-III-P reflects the integrity of the hepatic sinusoidal extracellular matrix.

Key words *N*-acetyl-β-glucosaminidase, liver preservation · Preservation, liver, rodents

Introduction

In spite of major advances in various aspects of orthotopic liver transplantation (OLT), primary graft nonfunction remains a devastating complication with a reported incidence of 5 %–15 %, which is fatal without urgent retransplantation [6, 9]. In order to maximize the use of the limited number of available liver allografts, it is extremely important to avoid primary graft nonfunction by accurate assessment of graft viability before actual transplantation. *N*-acetyl-β-glucosaminidase (β-NAG) is a lysosomal enzyme involved in the breakdown of proteoglycans [17]. In the liver, the activity of β-NAG in nonparenchymal cells is reported to be seven to eight times higher than that in parenchymal cells [16]. β-NAG is selectively taken up by a specific glycoprotein recognition system on reticuloendothelial cells such as Kupffer cells [1]. Type III procollagen peptide (P-III-P) is an amino-terminal peptide that is synthesized in parenchymal cells, cleaved during the conversion of type III procollagen into collagen, and released into the vascular bed [12]. Circulating P-III-P molecules are taken up by receptor-mediated endocytosis on endothelial cells [21] and either degraded or deposited in Disse's spaces [8]. The aim of this study was to clarify the significance of β-NAG and P-III-P in the vascular effluent of hepatic grafts as indicators of allograft injury.

Materials and methods

Inbred male Lewis rats weighing 200–250 g were used for the experiments. The rats were anesthetized with pentobarbital sodium (50 mg/kg i. p.) and given 500 units of heparin i. v. The liver was harvested by in situ perfusion through the terminal aorta with 20 ml of chilled, lactated Ringer's solution at 4 °C and then bathed in the same solution at the same temperature.

The livers were divided into five groups according to static cold preservation time as follows:

Table 1 Liver weight and harvesting time. Values are expressed as mean ± SD

Group	Liver weight (g)	Harvesting time (min)
1 (n = 10)	10.15 ± 0.30	15.1 ± 1.3
2 (n = 10)	10.15 ± 0.35	14.4 ± 1.3
3 (n = 10)	9.86 ± 0.36	15.1 ± 2.9
4 (n = 10)	9.85 ± 0.47	14.2 ± 1.0
5 (n = 10)	9.95 ± 0.39	14.3 ± 1.6

Group 1 (n = 10): 4 h of preservation only
Group 2 (n = 10): 4 h of preservation followed by 15 min of rewarming
Group 3 (n = 10): 6 h of preservation only
Group 4 (n = 10): 6 h of preservation followed by 15 min of rewarming
Group 5 (n = 10): minimal preservation only (approximately 30 s).

The preservation time of each group was determined on the basis of findings from our previous report on rat OLT in which hepatic grafts preserved for 4 h in chilled, lactated Ringer's solution (4 °C) were viable, while those preserved for 6 h were not [10].

Rewarming of the hepatic graft was achieved by placing the liver orthotopically in a separately prepared, totally hepatectomized animal. The rewarming time of 15 min was based on the actual time required for implantation in rat OLT [10].

After the assigned period of cold preservation with or without rewarming, the vascular effluent of the liver graft was obtained from the suprahepatic vena cava by flushing the portal vein with 5 ml of Ringer's solution (4 °C) [22].

The effluent glutamic-pyruvic transaminase (GPT) and the lactic dehydrogenase (LDH) concentrations were determined as described elsewhere [22]. P-III-P was measured with a radioimmunoassay kit (RIA-gnost P-III-P, Hoechst, Tokyo, Japan). β-NAG was measured using p-nitrophenyl-*N*-acetyl-β-glucosaminide substrate [24].

After the assigned period of cold preservation or rewarming, the liver specimens were taken and fixed with 10 % formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

All data were expressed as mean ± SD and were analyzed using Wilcoxon's rank sum test. P values less than 0.05 were taken as significant.

Results

The liver weight and harvesting time were comparable among the groups studied (Table 1).

Figure 1 shows the effluent GPT level of each group. The values of groups 2 and 4 were significantly higher than those of groups 1 and 3, respectively ($P < 0.01$ each), while group 4 had significantly higher values than group 2 ($P < 0.01$).

Figure 2 depicts the effluent LDH level among the five groups studied. The LDH level of group 4 was significantly higher than that of group 3 ($P < 0.01$), and group 2 had higher values than group 1, while groups 2 and 4 had comparable values.

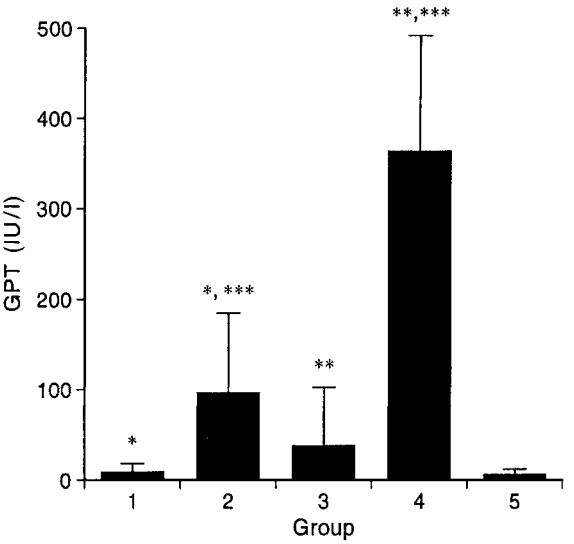

Fig. 1 Comparison of vascular effluent glutamic-pyruvic transaminase (GPT) concentrations. Rewarming increased GPT values (* $P < 0.01$ for group 2 vs group 1; ** $P < 0.01$ for group 4 vs group 3; *** $P < 0.01$ for group 4 vs group 2)

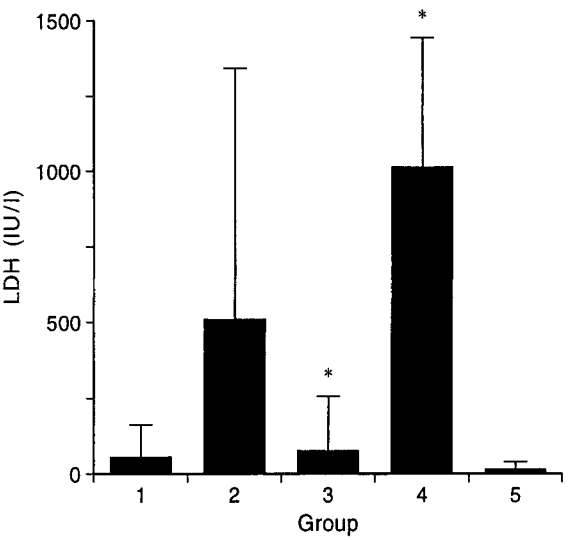

Fig. 2 Comparison of vascular effluent lactic dehydrogenase (LDH) concentrations ($P < 0.01$ for group 4 vs group 3)

Figure 3 depicts the effluent β-NAG level among the five groups studied. The values of groups 2 and 4 were significantly higher than those of groups 1 and 3, respectively ($P < 0.01$ each), while group 4 had significantly higher values than group 2 ($P < 0.05$).

Figure 4 demonstrates the effluent P-III-P level of each group. The values of groups 2 and 4 were comparable to those of groups 1 and 3, respectively, while the value of group 4 was significantly higher than that of group 2 ($P < 0.01$).

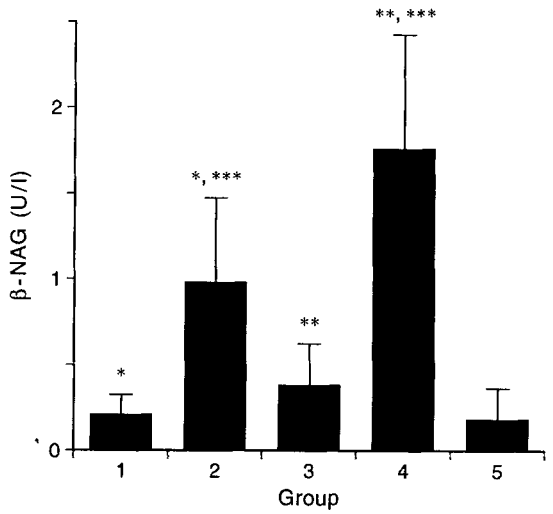

Fig. 3 Comparison of the vascular effluent N-acetyl-β-glucosaminidase (β-NAG) concentrations. Rewarming increased β-NAG values (* $P < 0.01$ for group 2 vs group 1; ** $P < 0.01$ for group 4 vs group 3;*** $P < 0.05$ for group 4 vs group 2)

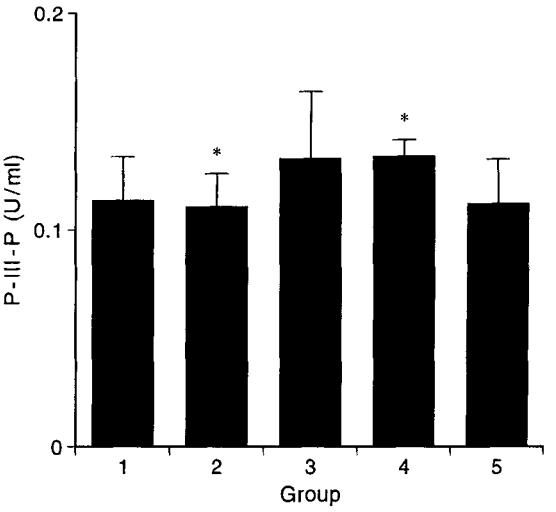

Fig. 4 Comparison of vascular effluent type III procollagen peptide (P-III-P) concentrations. Rewarning did not increase P-III-P levels. (P = ns for group 2 vs group 1 and for group 4 vs group 3). However, group 4 was significantly higher than group 2 (*$P < 0.01$)

Figure 5 exhibits photomicrographs of each group. Compared to groups 1 and 3, groups 2 and 4 demonstrated prominent ischemic changes, such as ballooning and denucleation of hepatocytes, while groups 3 and 4 revealed marked detachment of endothelial cells.

Discussion

With regard to assessing hepatic graft viability before actual transplantation, we have already reported the usefulness of graft effluent analysis, such as ammonia and tissue-type plasminogen activator (tPA) concentrations [19, 20]. Nishida et al. [15] demonstrated in rodents that the level of purine compounds in the perfusate may be a sensitive index of graft viability, and Bronsther et al. [3] reported that hyaluronic acid (HA) in the vascular effluent is predictive of human allograft function before transplantation. In small bowel transplantation, Mueller et al. [14] concluded that the HA level in the vascular effluent correlates well with graft survival. The present report demonstrated that β-NAG and P-III-P concentrations in the hepatic graft effluent are also indicators of graft injury in the rodent liver.

McKeown et al. in 1988 [13] demonstrated that prolonged cold ischemia is associated mainly with endothelial cell damage, and Clavien et al. in 1992 [5] reported that cold preservation leads to rounding of the sinusoidal lining cells (SLC), which eventually slough into the sinusoidal lumen, while most SLC themselves remain viable, as assessed by the trypan blue exclusion test. We also reported in 1992 [10] in rodent liver transplantation that the main site of injury in warm ischemia is the hepatocytes, whereas cold ischemia is associated with prominent endothelial cell damage. Recently, Arai et al. [2] demonstrated that activated hepatic macrophages may cause sinusoidal endothelial cell damage leading to hepatocyte necrosis in rats, while others reported that the Kupffer cells play an important role in graft viability [4]. With regard to rewarming injury, a prolonged period of rewarming leads to poor endothelial cell function [18].

In our present study, the β-NAG level in the vascular effluent was increased not only by prolonged cold preservation but also by rewarming. Yet, the P-III-P level in the vascular effluent was increased only by prolonged cold ischemia. In histological findings, prolonged cold ischemia was associated with predominant endothelial detachment, while rewarming led to ischemic change in parenchymal cells.

β-NAG is a lysosomal enzyme, the activity of which in nonparenchymal cells is seven to eight times higher than that in parenchymal cells [16], and the activation of Kupffer cells is known to trigger the release of β-NAG, resulting in parenchymal cell damage [7]. Therefore, the increase in effluent β-NAG level during cold preservation and rewarming may depend on the total damage to the hepatic graft. β-NAG is selectively taken up by the reticuloendothelial system [1], and the serum β-NAG level has been reported to be a sensitive indicator of both parenchymal cell damage and reticuloendothelial function in canine OLT [23].

At the same time, circulating P-III-P is reported to be either removed from circulation by receptor-mediated endocytosis on endothelial cells [21] or mainly deposited in Disse's spaces [8]. Since prolonged cold preservation causes the detachment of endothelial cells into si-

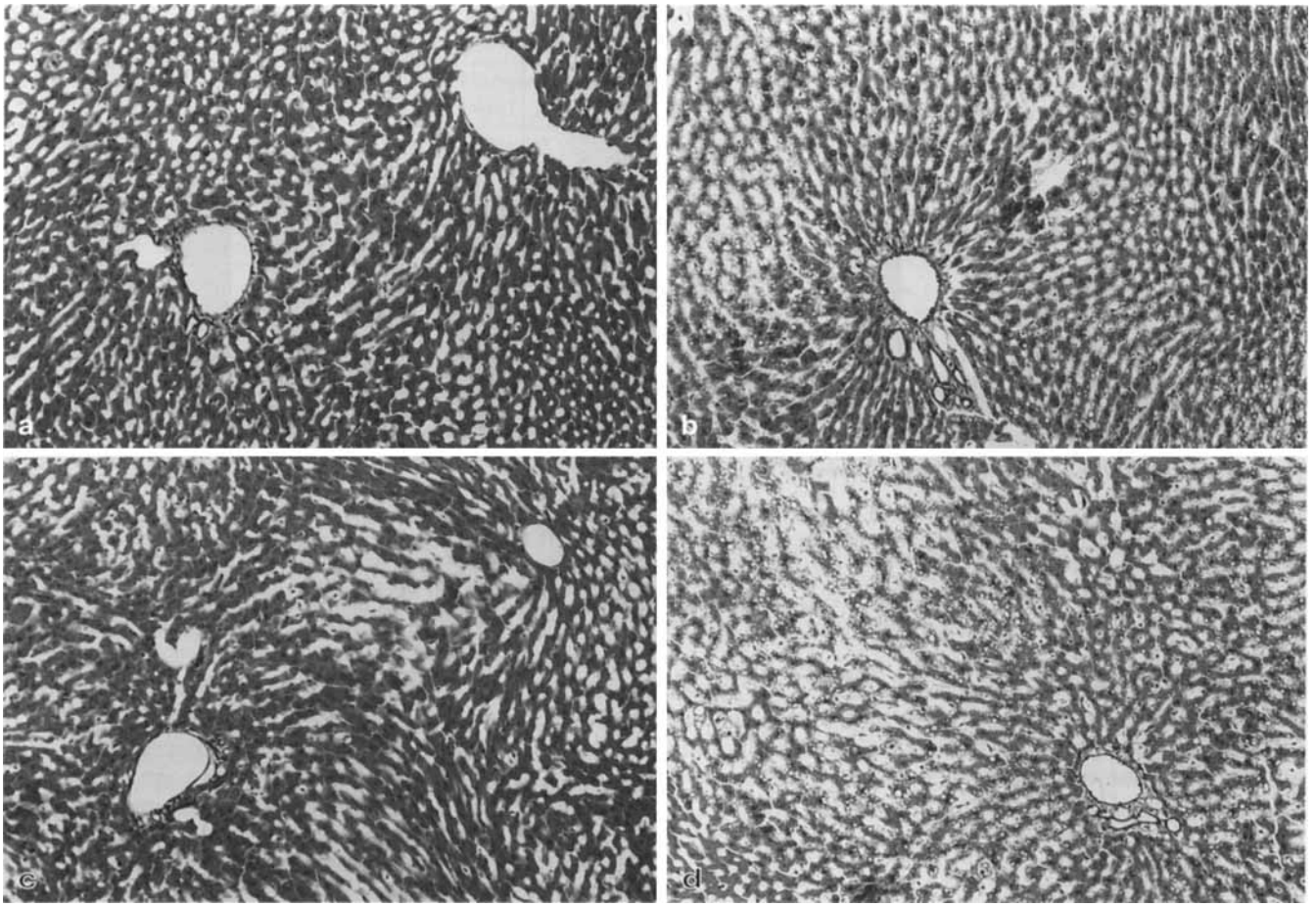

Fig. 5 a–d Photomicrographs of the livers (H&E, × 40): **a** group 1, 4 h of preservation only. Ischemic changes, such as hepatocyte and endothelial cell damage, are minimal; **b** group 2, 4 h of preservation followed by rewarming. Compared to group 1, hepatocyte damage, such as ballooning and denucleation, is prominent, while endothelial cell damage, such as the detachment of endothelial cells, is minimal; **c** group 3, 6 h of preservation only. Compared to group 1, the detachment of endothelial cells is prominent, while hepatocyte damage is almost the same as in group 1; **d** group 4, 6 h of preservation followed by rewarming. Hepatocyte and endothelial cell damage is prominent compared to the three other groups

nusoidal lumen and, as a result, endothelial damage [5], it is suggested that the increased effluent P-III-P level may depend on the degree of endothelial cell and extracellular matrix (ECM) damage to the hepatic graft. It has recently been acknowledged that ECM plays an important role in the attachment and communication among various types of cells [11].

In this study, we used chilled, lactated Ringer's solution (4 °C) as the preservation solution. Since UW solution is exclusively used in clinical liver transplantation, it is not clear whether the results of this study can be extrapolated to clinical OLT. However, since the mechanism of cold preservation-reperfusion injury in the rodent hepatic graft preserved in lactated Ringer's solution has been well documented [10], we believe that the β-NAG and P-III-P concentrations in the hepatic graft effluent may be reliable parameters of hepatic graft injury in OLT.

We have previously reported the usefulness of ammonia and tissue-type plasminogen activator (tPA) concentrations in the graft effluent for the prevention of primary graft nonfunction [19, 20]. In the present study, however, the measurement of β-NAG and P-III-P was performed at the end of the rewarming period to evaluate the influence of rewarming. Therefore, such parameters do not allow for a pretransplant assessment of hepatic graft viability.

In conclusion, the β-NAG concentration in the hepatic graft effluent seems to serve as a parameter for both parenchymal and nonparenchymal cell damage, while P-III-P in the effluent may be an indicator of cold ischemic injury.

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