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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver weight (g)</th>
<th>Harvesting time (min)</th>
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</thead>
<tbody>
<tr>
<td>1 (n = 10)</td>
<td>10.15 ± 0.30</td>
<td>15.1 ± 1.3</td>
</tr>
<tr>
<td>2 (n = 10)</td>
<td>10.15 ± 0.35</td>
<td>14.4 ± 1.3</td>
</tr>
<tr>
<td>3 (n = 10)</td>
<td>9.86 ± 0.36</td>
<td>15.1 ± 2.9</td>
</tr>
<tr>
<td>4 (n = 10)</td>
<td>9.85 ± 0.47</td>
<td>14.2 ± 1.0</td>
</tr>
<tr>
<td>5 (n = 10)</td>
<td>9.95 ± 0.39</td>
<td>14.3 ± 1.6</td>
</tr>
</tbody>
</table>

Group 1 (n = 10): 4 h of preservation only
Group 2 (n = 10): 4 h of preservation followed by 15 min of re-warming
Group 3 (n = 10): 6 h of preservation only
Group 4 (n = 10): 6 h of preservation followed by 15 min of re-warming
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), while groups 2 and 4 had comparable values.

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).