ABSTRACT

Reactive oxygen species (ROS) play a central role in ischemia–reperfusion injury after organ transplantation. They are degraded by endogenous radical scavengers such as antioxidant enzymes. The purpose of this study was to evaluate the temporal variations of antioxidant enzyme activities in liver transplant recipients. The study was performed in 13 liver transplant patients (11 men and 2 women). Blood samples were obtained pre- and postsurgical intervention: before transplant (T₀), and 1, 6, 12, 24, 48, and 72 hours, as well as 5 and 7 days thereafter. We determined total and specific superoxide dismutase (SOD) activity, catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR) activities as well as malondialdehyde (MDA) and low-density lipoproteins (LDL). The results showed increased SOD and mainly GPX activities after liver transplantation, which correlated with MDA levels. Total SOD activity was mainly represented by Mn-SOD (75%) and Cu,Zn-SOD (25%), whereas Fe-SOD was not detected. In conclusion, the enhanced antioxidant enzyme activities reported in this study indicated a control of oxidative stress generated in liver transplantation. In this sense, although MDA levels showed an enormous increase at 1 hour after transplantation, the lipid peroxidation was compensated for by GPX activity.

DURING ORTHOTOPIC human liver transplantation (OLT) increased levels of reactive oxygen species (ROS) have been observed to correlate with the presence of cytolysis. Nevertheless, the role of oxidative stress in causing or amplifying postreperfusion damage has not been conclusively defined, although it is generally accepted that ROS contribute to hepatic ischemia/reperfusion injury. The most relevant ROS toxic for cells are superoxide radicals (O₂⁻), hydroxyl radicals (HO⁻), and singlet oxygen (¹O₂). Their possible excess is controlled by several antioxidant enzymes as major defense mechanisms: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR). SOD, a metalloenzyme that converts O₂⁻ to hydrogen peroxide, occurs in three molecular forms that contain manganese (Mn), iron (Fe) or copper plus zinc (Cu, Zn) as prosthetic metals. CAT is an enzyme that detoxifies hydrogen peroxide. GPX catalyzes hydroperoxide decomposition to the stable form of hydroxides, specifically using reduced glutathione as the electron provider. In this context, glutathione participates in a variety of metabolic processes, transport, and detoxification, which protect against free radical damage. This process generates reduced glutathione (GSH) and oxidized glutathione (GSSG), the relative proportions of which reflect the capacity of the cell to cope with oxidative attack. GR catalyzes the reduction of GSSG to GSH using NADPH resulting from the pentose phosphate pathway. Peroxidation of unsaturated lipids in membranes is generally accepted as a major mechanism leading to cell lysis. Malondialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red specie absorbing at 535 nm. In the present study, 13 liver transplant recipients were studied before as well as 1, 6, and 12 hours as well as 1, 2, 3, 5, and 7 days after...
Antioxidant enzyme activities and malondialdehyde levels in liver transplant recipients (n = 13).

<table>
<thead>
<tr>
<th>Time</th>
<th>SOD (U/mg)</th>
<th>CAT (kU/mg)</th>
<th>GPX (nmol/mg)</th>
<th>GR (nmol/mg)</th>
<th>MDA (pmol/mg LDL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>175.8</td>
<td>55.2</td>
<td>2.32</td>
<td>4.15</td>
<td>138.81</td>
</tr>
<tr>
<td>T1h</td>
<td>225.86</td>
<td>22.52</td>
<td>2.72</td>
<td>4.21</td>
<td>420.00</td>
</tr>
<tr>
<td>T6h</td>
<td>255.90</td>
<td>23.70</td>
<td>2.78</td>
<td>4.28</td>
<td>599.75</td>
</tr>
<tr>
<td>T12h</td>
<td>275.35</td>
<td>26.10</td>
<td>2.86</td>
<td>4.35</td>
<td>1083.81</td>
</tr>
<tr>
<td>T1d</td>
<td>295.84</td>
<td>28.50</td>
<td>2.90</td>
<td>4.46</td>
<td>1083.81</td>
</tr>
<tr>
<td>T2d</td>
<td>309.50</td>
<td>29.00</td>
<td>2.92</td>
<td>4.48</td>
<td>1083.81</td>
</tr>
<tr>
<td>T3d</td>
<td>325.00</td>
<td>30.00</td>
<td>2.93</td>
<td>4.48</td>
<td>1083.81</td>
</tr>
<tr>
<td>T5d</td>
<td>335.00</td>
<td>30.00</td>
<td>2.93</td>
<td>4.48</td>
<td>1083.81</td>
</tr>
<tr>
<td>T7d</td>
<td>345.00</td>
<td>30.00</td>
<td>2.93</td>
<td>4.48</td>
<td>1083.81</td>
</tr>
</tbody>
</table>

Data are means ± SE of three values for each determination.

**RESULTS**

SOD activity was enhanced at 1 hour after transplantation relative to the value before transplantation (T₀). The activity returned to pretransplant values at 1 day after the liver transplantation to assess the time-course of MDA and these enzymes.

**MATERIALS AND METHODS**

The 13 adult patients (43 to 57 years old) included 11 men and 2 women. Written informed consent was obtained from the patients’ relatives. The study protocol was approved by the local Clinical Research (Ethics) Committee. Blood samples containing EDTA as anticoagulant were obtained pre- and postsurgical intervention before transplant (T₀), and 1, 6, and 12 hours, as well as 1, 2, 3, 5, and 7 days after liver transplantation. We determined total and specific SOD activity, CAT, GPX, and GR activities as well as MDA and low-density lipoproteins (LDL). To determine enzyme activities, cells were separated from plasma by centrifugation (1800 rpm/10 minutes). Plasma was recovered and cells washed with 50 mmol potassium phosphate buffer pH 7.8.

Total SOD activity was estimated by the ferricytchrome c method using xanthine/xanthine oxidase as the source of superoxide radicals; one unit of activity was defined according to McCord and Fridovich. SOD activity was also determined using nondenaturing (native) polyacrylamide gel electrophoresis (7.5% Tris-HCl gels) in a Criterion system (Bio-Rad, Richmond, Va, USA), where SOD activity was assayed using an in situ staining technique described by Beauchamp and Fridovich. The different types of SOD were differentiated by performing activity stains in gels previously incubated at 25 °C for 30 minutes in 50 mmol of potassium phosphate buffer, pH 7.8, containing either 2 mmol of potassium cyanide (KCN) or 5 mmol of H₂O₂. Cu,Zn-SODs are inhibited by KCN and H₂O₂; Fe-SODs are resistant to KCN but inactivated by H₂O₂; and Mn-SODs are resistant to both inhibitors. CAT activity was measured as described by Aebi. The decomposition of hydrogen peroxide was monitored by measuring the decrease in absorbance at 240 nm.

GPX activity was determined by spectrophotometry according with the method described in St Clair and Chow. Basically, this method reports the decrease in absorbance at 340 nm due to NADPH oxidation in the presence of an excess of GR. GR activity was determined according to Kang et al. Protein content was determined according to Lowry et al. using bovine serum albumin (BSA) fraction V as a standard. The level of lipid peroxidation products was expressed by malondialdehyde (MDA) content according to Buege and Aust. Plasma obtained as previously described was treated with 0.25% thiobarbituric acid in 10% trichloroacetic acid. After heating at 95 °C for 30 minutes the mixture was cooled in ice and centrifuged (12,000 × g/10 minutes). The absorbance of the supernate was read at 535 nm and corrected for nonspecific turbidity by subtracting the value at 600 nm. The blank was 0.25% thiobarbituric acid in 10% trichloroacetic acid. The concentration of MDA was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹. LDL rich in triglycerides are synthesized in the liver. They were measured following an automated method in an Hitachi-912 analyzer (Roche).

Analysis of variance (ANOVA) was performed. When significant differences occurred, mean separation was performed using the least significant difference (P = .05) method. Data are presented as mean values ± standard errors.
procedure, increasing again from day 2 (Table 1). The pattern of SOD activity before and after liver transplantation was followed by staining non-denaturing (native) polyacrylamide gels for SOD activity. The activity was mainly represented by Mn-SOD (75%) and Cu,Zn-SOD (25%), whereas Fe-SOD was not detected (Fig 1). CAT activity showed a similar pattern to total SOD activity but reached a maximum at 6 hours after transplantation (see Table 1).

GPX activity was clearly enhanced at 5 days after transplantation (see Table 1). In contrast, GR was slightly increased at 1 hour after transplantation and then reduced during the study. MDA levels showed an enormous increase at 1 hour after transplantation with enhanced values maintained until day 5 after transplantation when they started to decrease (see Table 1).

**DISCUSSION**

The results obtained herein confirmed the presence of an oxidative stress. The enhanced SOD activity represents the response of the organism toward superoxide radicals generated during and after liver transplantation. A consequence of this activity is the generation of hydrogen peroxide, which is decomposed by CAT activity. Lipid peroxidation is the principal damage caused by ROS. MDA, one of the final products in the peroxidation process, serves as an indication of the amount of generated ROS. We have shown high levels of MDA, although they decreased upon a maximum of GPX activity at day 5 after transplantation.

The decreased GR activity seemed to support the hypothesis that some patients display a blockade of the pentose phosphate pathway, as previously noted by De Vega et al.\(^5\) and López Barea et al.\(^12\). The action of GR requires NADPH and regenerates GSH. It may be possible that glucose-6-phosphate-dehydrogenase, an enzyme that catalyzes the first step in the pentose phosphate pathway and whose activity is regulated by the concentration of NADPH, might be blocked in this setting.
REFERENCES


