RED CELL SUPEROXIDE DISMUTASE IN PATIENTS WITH DIFFERENT LIVER DISEASES

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ABSTRACT

Superoxide dismutase (SODs, superoxide oxidoreductase, E.C. 1.15.1.1.) activity has been estimated in red blood cells from patients suffering from liver cirrhosis and hepatocellular carcinoma. Erythrocytes superoxide dismutase activity from patients with liver cirrhosis was found to be significantly higher than normal, while its activity was not significantly raised in patients with hepatocellular carcinoma. These observations reflect overproduction of superoxide radicals which leads to increased SOD activity. If the liver tissues reach the hepatocellular carcinoma stage subsequent damage of mitochondrial membranes, a lack of \( \mathrm{O}_2 \) production occurs, therefore a lack of mitochondrial enzymes, including SOD occurs. Accordingly, the assay of SOD can be used to follow-up malignant transformation of liver tissues.

INTRODUCTION

The recent advances in immunology provided important information for the characterization of different liver disorders. However, enzyme assay is another area of progress in liver disorders. The interest in the study of enzyme marker is not only due to technical improvements but also to the involvement of enzyme assay which combines traditional and the more recently developed techniques in liver diseases. Superoxide dismutases (SODs) are a family of enzymes which their substrate is unstable toxic free radical produced as a by-product of oxidative metabolism. These enzymes protect cells by catalyzing the dismutation of the superoxide radical (\( \mathrm{O}_2^- \)) to hydrogen peroxide (\( \mathrm{H}_2\mathrm{O}_2 \)) (1).

\[
2\mathrm{O}_2^- + 2\mathrm{H}^+ \rightarrow \mathrm{H}_2\mathrm{O}_2 + \mathrm{O}_2
\]

The liver has high levels of SODs enzymes, cytosolic Cu-Zn-SOD and mitochondrial Mn-SOD. Several investigators reported a biochemical changes of SOD in liver and gastrointestinal tissues reflecting the abnormalities of superoxide radicals, in rat ascites hepatoma cells (2), human fibroblasts and hepatoma cells (3), cultured...
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gastric mucosal cells (4), rat liver during development (5), cultured rat liver slices (6), colon cancer (7) and in hepatocellular carcinoma tissues (8,9).

MATERIAL AND METHODS

All chemicals used were of analar or equivalent grade and were obtained from Sigma Chemical Company.

This study was carried out on twenty-five patients of different ages and sexes suffering from liver cirrhosis and five patients with hepatocellular carcinoma. These patients were selected from the Gastroenterology Center, Mansoura University. For comparison, ten healthy persons were considered as a control group. Extraction of the red cell superoxide dismutase with ethanol-chloroform according to the method of Winterbourn et al (10). The superoxide dismutase present in the extraction was assayed by the method of Nishikimi et al (11). The reaction was initiated by the addition of phenazine methosulphate (PMS) and the ability of the enzyme to inhibit the PSM-mediated reduction of nitroblue tetrazolium (NBT) dye, and the increase in the absorbance at 540 nm due to the formation of the dye was recorded. Results were expressed as units of superoxide dismutase per gram of hemoglobin and one unit is defined for a particular system as that amount of enzyme causing half the maximum inhibition of NBT reduction. Purified superoxide dismutase was shown to inhibit the initial rate of the phenazine methosulphate-induced reduction of NBT (0.5 μg of the purified enzyme gives 80% inhibition).

RESULTS

As shown from Table (1), superoxide dismutase levels in red blood cells isolated from cirrhosis patients (5.33 ± 2.05 units/g Hb) were significantly higher (p < 0.05) than those in normal values (3.83 ± 1.17 unit/g Hb). No significant changes of the red blood cells superoxide dismutase isolated from hepatocellular carcinoma patients when compared with control values Fig. (1). On the other hand, the percent inhibition of nitroblue tetrazolium confirmed the obtained results which are presented in a scatter diagram (Fig. 2).

Table (1) : Superoxide dismutase activity (units/g Hb) and percent inhibition of NBT in red blood cells of patients with liver cirrhosis and hepatocellular carcinoma (HCC) as well as normal control subjects.

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<tr>
<th></th>
<th>Control</th>
<th>Cirrhosis</th>
<th>HCC</th>
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<tbody>
<tr>
<td>SOD Activity (units/g Hb)</td>
<td>Mean ± SD (n)</td>
<td>3.83 ± 1.17 (10)</td>
<td>5.33 ± 2.05 (25)</td>
</tr>
<tr>
<td></td>
<td>t</td>
<td>1.9231</td>
<td>0.6531</td>
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<tr>
<td></td>
<td>p</td>
<td>&lt;0.05</td>
<td>0.25</td>
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<tr>
<td>% inhibition of NBT reduction</td>
<td>Mean ± SD (n)</td>
<td>38.8 ± 10.94 (10)</td>
<td>50.7 ± 14.5 (25)</td>
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<tr>
<td></td>
<td>t</td>
<td>1.968</td>
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<td></td>
<td>p</td>
<td>&lt;0.05</td>
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DISCUSSION

Over the past years there has been a great deal of new information about oxygen radical induced cellular injury. Oxygen metabolities play an important role in many different biological processes, including inflammation (12,13), oxygen induced lung injury (14), ischemic reperfusion injuries (15), aging (16) and carcinogenesis (17,18).

Patients with cirrhosis have portal hypertension and portal-systemic shunting and additionally shunts within the liver are likely to occur. Liver cell function is likely to be impaired to a variable degree, and there is a risk of hepatocellular carcinoma to occur probably related to the promoting effect of increased liver-cell division. The reasons for the liver cell dysfunction in cirrhosis are complex, one of these is probably due to over production of large amount of hydrogen peroxide (H₂O₂), which could potentially enhance neoplastic transformation by augmented both genetic instability of the cells (19,20) and its capacity to injure and penetrate host tissues (21). The function of superoxide dismutase (SOD) with the red cells is probably protective, preventing the fast reactive oxygen O₂⁺ from damaging the liver cell itself. With respect to mortality, some studies clearly show that animals pretreated with SOD survive the hypertensive challenge better (22).

The SODs is an inducible enzyme, assuming that the substrate of this enzyme, superoxide radical is responsible for the induction of the enzyme. We have demonstrated that the reason for the high SOD activity
Fig. (1) : Erythrocytes superoxide dismutase activity levels in liver cirrhosis, hepatocellular carcinoma (HCC) patients and control subjects.

Fig. (2) : Scatter diagram of erythrocyte superoxide dismutase activity levels in liver cirrhosis, hepatocellular carcinoma (HCC) patients and control subject.
in liver cirrhosis with no significant elevation in hepatocellular carcinoma, may be firstly due to a large quantity of \( \text{O}_2^- \) produced in cirrhosis while in hepatocellular carcinoma the mitochondria was not able to produce \( \text{O}_2^- \). Also the production of \( \text{H}_2\text{O}_2 \) may participate in the damage of the liver mitochondrial membrane, consequently the excretion of mitochondrial SOD is decrease. The highly damaged mitochondria of the fast- and medium growing tumours explain the decreased activity of mitochondrial SOD and the normal flux of \( \text{O}_2^- \) by tumour cells remains unclear.

The result of the present study demonstrated that the SOD activity in red cells of patients with liver cirrhosis showed significant increase, whereas in the group of patients with hepatocellular carcinoma no significant changes were observed when compared with normal values. This finding is in agreement with the result of Huang (8) and Huang and Wu (9) who observed low activities of total SODs in hepatocellular carcinoma, and this may be a contributing factor that leads to impairment of SOD activity. Finally, the assay of SOD may be useful to follow-up malignant transformation of liver tissues.

REFERENCES


