Superoxide Dismutase in Various Tissues from Rat Carcinoma in the Maxillary Sinus

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ABSTRACT

Distribution profiles of superoxide dismutase isoenzymes in various tissues of rabbits with the Vx-2 carcinoma in the maxillary sinus were compared with those of control rabbits. Copper- and zinc-containing superoxide dismutase (Cu,Zn-SOD) activity in the liver of rabbits decreased significantly 3 weeks after transplantation. Manganese-containing superoxide dismutase (Mn-SOD) activity did not decrease significantly within 5 weeks after transplantation. In other tissues from the tumor-bearing rabbits, Cu,Zn-SOD and Mn-SOD activities were not changed within 5 weeks. No Mn-SOD activity and low Cu,Zn-SOD activity were detected in the Vx-2 carcinoma.

These results suggest that the Vx-2 carcinoma has lost most of its ability to defend against oxygen toxicity and this ability decreased only in the liver of rabbits bearing the Vx-2 carcinoma in the maxillary sinus.

INTRODUCTION

SOD³ (EC 1.15.1.1) is an enzyme whose function is to protect against the potentially damaging reactivities of the superoxide radial (O₂⁻) generated by aerobic metabolic actions (8). Two types of SOD have been found in all mammalian cells except erythrocytes. Cu,Zn-SOD was present in both the cytosol and the intermembrane space of the mitochondria, and Mn-SOD was present in the mitochondrial matrix (5). Several studies have been made on the SOD activities of tumor cells which usually have lowered levels of both Cu,Zn-SOD and Mn-SOD (10). However, there is little information concerning SOD activities in tissues from tumor-bearing animals. In the present study, we examined the distribution profiles of SOD isoenzymes in the Vx-2 carcinoma and various tissues from Vx-2 carcinoma-bearing rabbits.

MATERIALS AND METHODS

Xanthine, NBT, and O-dianisidine were purchased from Nakarai Chemical Co. (Kyoto, Japan), xanthine oxidase from Sigma Chemical Co. (St. Louis, Mo.), and riboflavin from Tokyo Chemical Co. (Kyoto, Japan). Other reagents were of the highest purity available. Male rabbits (2.0 to 2.5 kg) of the Japanese white strain were used. Animals were individually caged, maintained in a 12-hr dark/12-hr light cycle at about 22°, and fed laboratory chow and water ad libitum. Other reagents were of the highest purity available. Male rabbits (2.0 to 2.5 kg) of the Japanese white strain were used.

Animals were individually caged, maintained in a 12-hr dark/12-hr light cycle at about 22°, and fed laboratory chow and water ad libitum. The Vx-2 carcinoma cells were prepared by the standard procedure of Evance et al. (4), and about 1.5 × 10⁶ cells were injected into the maxillary sinus of rabbits. The Vx-2 carcinoma is a transplantable anaplastic-type carcinoma of the rabbit which is derived from cutaneous papillomas of rabbits induced by Shope virus (13). Tissue extracts from rabbits were prepared as follows. Animals were killed by stunning and bleeding at 2 to 5 weeks after the transplantation, and tissues (brain, liver, kidney, heart, lung, spleen, stomach, small intestine, masseteric muscle, tongue, submaxillary gland, gingiva, mucous membrane of maxillary sinus) were quickly removed. All of the following procedures were carried out at about 0 °C. Tissues were rinsed with 0.25 M sucrose, minced with scissors, and homogenized in 5 volumes of 5 mM potassium phosphate buffer, pH 7.5, in a Waring blender. Each homogenate was sonicated at 10 kHz for 30 sec and centrifuged at 105,000 × g for 30 min, and the precipitate was discarded.

The SOD activity was assayed by the standard method of Beaufay and Fridovich (1). The reaction mixture (1 ml) contained 0.1 mM xanthine, 0.025 mM NBT, 0.1 mM EDTA, 0.06 M sodium carbonate buffer (pH 10.2), and xanthine oxidase. The rate of reduction of NBT was measured at 560 nm. The concentration of xanthine oxidase was adjusted so that the absorbance change was 0.0165/min without SOD. One unit of SOD was defined as the amount of enzyme that inhibits 50% of the rate of NBT reduction at room temperature. Cu,Zn-SOD was assayed after chloroform/ethanol treatment of the sample to inactivate Mn-SOD (14). Mn-SOD was assayed in the presence of 8 mM KCN to inhibit Cu,Zn-SOD activity (14).

Polyacrylamide gel electrophoresis was performed by the method of Davis (2) with 10% gels using 10 mM Tris/glycine (pH 8.7). The SOD activity in the gel was detected by the method of Misra and Fridovich (9). Protein was measured by the method of Lowry et al. (7) with bovine serum albumin as standard. Statistical significance of difference between mean values was assessed by Student's t test.

RESULTS AND DISCUSSION

Distribution profiles of SOD isoenzymes in various tissues of rabbits with the Vx-2 carcinoma in the maxillary sinus were compared with those of control rabbits (Table 1). The results for control rabbits were similar to those from rats (11, 12) and humans (6) except for perilous tissues. There is no report on distribution of SOD isoenzymes in perilous tissues. Total and Cu,Zn-SOD activities in livers from the carcinoma-bearing rabbits were significantly low (p < 0.01) compared to those from control animals. In contrast, Mn-SOD activity was not changed. In other tissues from the tumor-bearing rabbits, the total activities due to Cu,Zn-SOD, and Mn-SOD were not significantly changed compared to those from control rabbits. The Vx-2 carcinoma itself showed very low total and Cu,Zn-SOD activities and no Mn-SOD activity.

Polyacrylamide gel disc electrophoresis of extracts of the Vx-2 carcinoma and of combined maxillary sinus and nasal mucous membrane and liver from control rabbits was performed, and the gels were stained for SOD activity (Fig. 1). Three activity bands were detected in the 2 extracts from the latter animals; the slowest-migrating band disappeared after chloroform/ethanol treatment of the extracts and the other 2 bands disappeared in the presence of 2 mM KCN. These results...
Table 1
Total, Cu,Zn-SOD, and Mn-SOD activities in extracts of various tissues from control and Vx-2 carcinoma-bearing rabbits

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (±)</td>
</tr>
<tr>
<td>Liver</td>
<td>927 ± 158</td>
</tr>
<tr>
<td>Kidney</td>
<td>476 ± 75</td>
</tr>
<tr>
<td>Heart</td>
<td>277 ± 82</td>
</tr>
<tr>
<td>Spleen</td>
<td>172 ± 41</td>
</tr>
<tr>
<td>Lung</td>
<td>166 ± 36</td>
</tr>
<tr>
<td>Stomach</td>
<td>153 ± 51</td>
</tr>
<tr>
<td>Small intestine</td>
<td>196 ± 33</td>
</tr>
<tr>
<td>Brain</td>
<td>166 ± 27</td>
</tr>
<tr>
<td>Masseter</td>
<td>192 ± 34</td>
</tr>
<tr>
<td>Submaxillary gland</td>
<td>67 ± 16</td>
</tr>
<tr>
<td>Tongue</td>
<td>255 ± 72</td>
</tr>
<tr>
<td>Gingiva</td>
<td>147 ± 38</td>
</tr>
<tr>
<td>Combined maxillary sinus and nasal mucous membrane</td>
<td>203 ± 48</td>
</tr>
</tbody>
</table>

Vx-2 carcinoma-bearing rabbits were killed 5 weeks after transplantation of the tumor in the maxillary sinus.

**Mean ± S.D. of 7 rabbits.**

**Significantly different from controls (p < 0.01).**

**ND, not detected.**

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**Fig. 1.** Polyacrylamide gel disc electrophoresis of crude extract of Vx-2 carcinoma (a) and of combined maxillary sinus and nasal mucous membrane (b) and liver (c) from control rabbits. Fifty µl (a, b) and 20 µl (c) of the extract were separately used. The gels were stained for SOD activity. The 2 extracts from control rabbits contained both Cu,Zn-SOD and Mn-SOD; the extract from the Vx-2 carcinoma contained only Cu,Zn-SOD.

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**Chart 1.** Time course of change in hepatic SOD activity after Vx-2 carcinoma transplantation. Rabbits were killed from 2 to 5 weeks after transplantation, and total, Cu,Zn-SOD, and Mn-SOD activities were assayed in the liver extract. Point, mean of 7 (control) and 3 (Vx-2 carcinoma-bearing) rabbits; bars, S.D.

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show that the slowest-migrating activity is Mn-SOD and the other 2 are Cu,Zn-SOD. Similar electrophoretic patterns were obtained with extracts of other tissues except for the Vx-2 from control and carcinoma-bearing rabbits (not shown). In the case of the Vx-2 carcinoma, the activity bands corresponding to Cu,Zn-SOD were detected, but the band corresponding to Mn-SOD was absent.

Chart 1 shows a time course of changes in total, Cu,Zn-SOD, and Mn-SOD activities in the liver of Vx-2 carcinoma-bearing rabbits. Both total and Cu,Zn-SOD activities decreased significantly 3 weeks after transplantation (p < 0.05). Mn-SOD activity did not decrease in this 5-week period.

In the present study, the Vx-2 carcinoma was found to have a lowered level of Cu,Zn-SOD and to lack Mn-SOD. The loss of Mn-SOD has been found in spontaneous, transplanted, virally induced, chemically induced, in vitro, and in vivo tumor cells (10). Furthermore, Cu,Zn-SOD activity in the liver was found to decrease after transplantation of the Vx-2 carcinoma. This is the first report on the decrease in hepatic SOD activity in...
tumor-bearing animals. This decrease might be similar to the classical depression of liver catalase activity accompanying certain extra-hepatic tumors (15, 16, 17). Dinescu-Romalo and Mihai (3) observed no difference in hepatic SOD activities between Guerin T₈ ascites tumor-bearing and normal rats. The present results suggest that the Vx-2 carcinoma lost most of its ability to defend against oxygen toxicity and this ability decreased only in the liver among those tissues examined in rabbits bearing the Vx-2 carcinoma in the maxillary sinus.

REFERENCES

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