Superoxide Dismutase, Catalase, and Glutathione Peroxidase in Red Blood Cells from Patients with Malignant Diseases

Renée Gonzales, Christian Auclair, Emmanuelle Voisin, Huguette Gautero, Didier Dhermy, and Pierre Boivin

ABSTRACT

Superoxide dismutase (SOD), catalase, and glutathione peroxidase activities have been determined in red blood cells isolated from patients with acute myelogenous leukemia, chronic lymphocytic leukemia, Hodgkin’s disease, lymphosarcoma, and various visceral cancers.

In all investigated cases, both catalase and glutathione peroxidase activities were found to be normal in all investigated cases. In the group of patients with visceral cancers, SOD activity was found to be normal as well. In contrast, SOD activity was found to be significantly increased in red blood cells from patients with acute myelogenous leukemia and lymphoproliferative syndromes. This increase in superoxide level was not related to either reticulocytosis or hypocromic anemia. No relationship was found between the SOD level and the stage, the extension of the disease, or the presence of an inflammatory syndrome. The highest SOD levels were observed in untreated patients or during the early time period of the treatment. SOD levels further decrease as a function of the increase in the duration of the treatment. These results suggest an abnormality in the regulation of the expression of the SOD gene in the pluripotent stem cells.

INTRODUCTION

SODs are enzymes the function of which is to increase the dismutation rate of the superoxide anion. In connection with catalase and the glutathione reductase-glutathione peroxidase system, these enzymes are thought to protect aerobic cells against oxygen toxicity. During the past few years, SOD activity in tumor cells has received increasing attention. In most cases, differences in the activity of SOD have been found between normal and malignant cells. This difference is always manifested as a lower manganese-containing SOD activity and usually but not always as lowered copper-containing SOD activity. Exceptions have been effectively found in the case of L1210 leukemia cells as well as in human leukemia cells. In those cells, copper-containing SOD activity was significantly higher as compared to normal cells of similar types. The present paper reports: (a) that an increase in copper-containing SOD activity is also observed in RBC of patients with acute myeloblastic leukemia, chronic lymphoblastic leukemia, Hodgkin’s disease, and lymphosarcoma; (b) that this abnormality is not observed in patients with visceral cancers; and (c) that in all cases catalase and glutathione peroxidase are normal.

MATERIALS AND METHODS

Blood Samples. Blood was collected in heparinized tubes from normal donors and patients and passed over cotton to remove WBC. The blood sample was then centrifuged and washed twice in 0.9% NaCl solution. After the last centrifugation, cold water was added to the packed blood cells to effect lysis.

SOD Activity Determination. To 1.88 ml of the blood lysate were added 0.8 ml of chloroform:ethanol (3:5, v/v) and 0.3 ml of water to precipitate hemoglobin. After centrifugation, SOD activity was determined in the supernatant. The SOD activity was measured by the inhibition of the reduction of nitroblue tetrazolium by superoxide anion (O2-) produced by potassium superoxide (K2O2) dissolved in dimethyl sulfoxide, as described by Henry et al. (5) with minor modifications. The assay mixture was composed of 0.05 M phosphate buffer, pH 8.60, 10^-4 M EDTA, and 10^-3 M hydrogen peroxide, at 22°. The release of oxygen was continuously recorded, and the total oxygen released after 2 min was determined. Under suitable conditions, the amount of oxygen released after 2 min is linear with respect to the amount of enzyme which liberates 1 nmol of oxygen in 2 min.

Catalase Activity Determination. Aliquots of chloroform:ethanol extract were subjected to various dilutions in 0.05 M phosphate buffer, pH 7.40. Each dilution was added in an oxygraph chamber containing 0.05 M phosphate buffer (pH 7.40), 10^-4 M EDTA, and 10^-3 M hydrogen peroxide, at 22°. The release of oxygen was continuously recorded, and the total oxygen released after 2 min was determined. Under suitable conditions, the amount of oxygen released after 2 min is linear with respect to the amount of enzyme which liberates 1 nmol of oxygen in 2 min.

Glutathione Peroxidase Activity Determination. Glutathione peroxidase activity was determined essentially according to the method of Paglia and Valentine (3, 9) with minor modifications. A unit of glutathione peroxidase activity is taken as the amount of enzyme which consumes 1 μmol NADPH/min.

Patients. Patients with acute myeloblastic leukemia, chronic lymphocytic leukemia, Hodgkin’s disease, lymphosarcoma, and various visceral cancers were randomly selected for this study without consideration for age, sex, therapy regimen, or stage of the disease. The diagnosis of the various diseases was established using standard clinical and biological criteria.

Statistical Analysis. Statistical analysis of all data was done using Student’s t test or the corrected χ^2 test of Yates. The significance of the correlation coefficient (r) was estimated using the following relationship:

\[ t_r = \frac{r}{\sqrt{1 - r^2}} \sqrt{n - 2} \]
RESULTS AND DISCUSSION

The results of enzyme analysis in RBC from different groups of patients are summarized in Table 1. All enzymatic activities are expressed in units per ml of blood per hematocrit \times 100. This mode of expression, used instead of the units per g of hemoglobin, avoids possible overestimations of enzymatic activities in the case of normocytic hypochromic anemia which is not rare in patients suffering from extensive neoplasia. Values indicated in Table 1 show first that, in all groups of patients, both catalase and glutathione peroxidase activities were in normal ranges as compared to the control group. In the group of patients with visceral cancers, SOD activity was found as well to be in a normal range as described previously (7). In contrast, in all groups of patients suffering from malignant diseases of hematological origin, SOD activity was found to be markedly higher as compared to the control group. It must be noted that, in each group, the values of SOD activity are highly dispersed. The individual values of SOD activity varied from 2.75 to 6.95 units in patients with lymphosarcoma, from 2.60 to 8.20 units in patients with Hodgkin’s disease, and from 2.90 to 7.50 units in patients with leukemia. Moreover, in patients with lymphosarcoma and leukemia, there are indications of a non-Gaussian distribution of SOD levels ($\chi^2 < \chi^2_{0.05}$) with a bimodal distribution of subnormal values and high values about the mean. In all groups of patients investigated, biological parameters such as hemoglobin concentration, reticulocyte count, and erythrocyte count were highly dispersed. These abnormalities could be responsible for significant changes in SOD level. We have therefore investigated the possible relationship which may exist between these different parameters and SOD activity.

Table 2 shows the different values of the correlation coefficient ($r$) obtained between SOD levels, hemoglobin concentration, erythrocyte count, and reticulocyte count. In all cases, these values are not statistically significant ($p > 0.05$). These observations indicate that the SOD concentration is not influenced by secondary factors and therefore suggest that the SOD level reflects in each group of patients an intrinsic increase of SOD activity in the RBC. This phenomenon is comparable to the one observed in patients suffering from trisomy 21 where the SOD level was always increased according to a gene dosage effect (10). It is of interest to note that the 1.47 trisomy/normal ratio of the mean values of SOD level in RBC is quite similar to those obtained in hematological malignant diseases which varied from 1.31 to 1.69. Among various exogenous factors which might affect the SOD level in cells is the nature of the chemotherapy regimen. In this way, we have reported previously that, in patients with myeloma and under nitrogen mustard therapy, the SOD level was highly dispersed with very low values in 2 cases associated with intravascular hemolysis (1, 2). Chart 1 shows the relationship which exists between the SOD level and the treatment of the patients. It appears that there is an inverse correlation ($r = -0.42$) between the SOD level and the duration of the treatment. If one considers 2 groups of patients, the first one composed of patients having either no treatment or a treatment of less than 6 months and the other one composed of patients having a treatment for more than 6 months, the SOD levels are, respectively, 5.52 ± 1.42 (S.D.) and 4.40 ± 0.89. These values are significantly different at $p < 0.05$. Since the duration of the treatment is directly related to the longevity of the disease, we have investigated the possible relationship which may exist between the SOD level and the stage of the various
Chronic lymphoblastic leukemia
Lymphosarcoma
Hodgkin's disease
to the stage of the disease. Two other parameters of the extent
diseases. Table 3 shows the different values of SOD levels. In
all cases, these values are not significantly different with respect
to the stage of the disease. Two other parameters of the exten-
sion of the disease have been considered: (a) dissemination to
bone marrow in lymphoproliferative disorders; and (b) occurrence
of an inflammatory syndrome. The results indicated in Tables 4
and 5 show that there is no relationship between SOD level and
these parameters.

The previous determination of SOD level in RBC of patients
suffering from various diseases such as hemochromatosis, sple-
nonegaly myeloid, Vaquez disease, and various visceral cancers
has indicated normal SOD activity in each group of patients (7).
It would appear that high values of SOD are often associated
with senility syndrome (7), whereas high values of SOD are
always observed in trisomy 21 (10). The present report shows
that the SOD level is also increased in RBC of patients suffering
from malignant diseases of hematological origin. It is unlikely
that this increase reflects an abnormality in the oxidative metabolism
of the cells, since both catalase and glutathione peroxidase are,
in all cases, in normal ranges. The absence of relationship
between the increase in SOD level and various biological and
clinical parameters suggests that this increase is the result of a
disturbed gene expression in the stem cells. In this way, it is of

interest to ascertain the effect of the chemotherapy leading to
the normalization of the SOD level.

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Table 3
SOD activity in RBC from patients at various stages of the diseases

<table>
<thead>
<tr>
<th>Stage</th>
<th>Lymphosarcoma</th>
<th>Hodgkin’s disease</th>
<th>Chronic lymphoblastic leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4.60±1.00</td>
<td>5.74±1.23</td>
<td>4.73±1.00</td>
</tr>
<tr>
<td>II</td>
<td>5.22±1.00</td>
<td>4.83±1.10</td>
<td>5.53±1.65</td>
</tr>
<tr>
<td>III</td>
<td>4.28±1.00</td>
<td></td>
<td>4.54±1.28</td>
</tr>
<tr>
<td>IV</td>
<td>4.58±0.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6 Numbers in parentheses, number of patients in each group.

" Mean ± S.D.

Table 4
SOD activity in RBC from patients with or without dissemination to bone marrow

<table>
<thead>
<tr>
<th>SOD activity (units/ml blood/hematocrit x 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With dissemination</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
</tr>
</tbody>
</table>

6 Numbers in parentheses, number of patients in each group.

" Mean ± S.D.

Table 5
SOD activity in RBC from patients with or without an inflammatory syndrome

<table>
<thead>
<tr>
<th>SOD activity (units/ml blood/hematocrit x 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With an inflammatory syndrome</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
</tr>
<tr>
<td>Acute myeloblastic leukemia, chronic myeloblastic leukemia</td>
</tr>
</tbody>
</table>

6 Mean ± S.D.

7 Numbers in parentheses, number of patients in each group.
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