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

Renée Gonzales,2 Christian Auclair,3 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 in normal ranges of activity. 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 hypochromic anemia. No relationship was found between the SOD level and the stage, the extension of the disease, or the 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

SODs4 are enzymes the function of which is to increase the dismutation rate of the superoxide anion (6). In connection with catalase and the glutathione reductase:glutathione peroxidase system, these enzymes are thought to protect aerobic cells against oxygen toxicity (4). 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 usually manifested as a lower manganese-containing SOD activity and usually not as lowered copper-containing SOD activity (8). Exceptions have been effectively found in the case of L1210 leukemia cells as well as in human leukemia cells (11,12). 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 10-4 M nitroblue tetrazolium; 100 µl of K2O2 containing dimethyl sulfoxide were added under stirring to the above mixture. The absorbance of each solution was read at 550 nm against a control in which pure dimethyl sulfoxide had been added. One unit of enzymatic activity is defined as the amount of enzyme causing 50% inhibition of the reduction to formazan observed in the blank.

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 oxigraph chamber containing 0.05 M phosphate buffer (pH 7.40), 10-4 M EDTA, and 10-3 M hydrogen peroxide, at 22°C. 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 various chloroform:ethanol extract dilutions. A unit of catalase activity is taken as 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 sex, age, therapy regimen, or stage of the disease (Service d'Hématologie Clinique, Hôpital Beaujon, Pr. P. Boivin). 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 x2 test of Yates. The significance of the correlation coefficient (r) was estimated using the following relationship:

\[ t = \frac{r}{\sqrt{1 - r^2}} \sqrt{n - 2} \]

1 This research was supported by Grant CRL 79.5.010.2 from Institut National de la Santé et de la Recherche Médicale. 2 Present address: Institute of Hematology, William Soler Hospital, Altahabana, Cuba. 3 To whom requests for reprints should be addressed, at Institut Gustave Roussy, 94800 Villejuif, France. 4 The abbreviation used is: SOD, superoxide dismutase.
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 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 variations indicate that the SOD concentration is not influenced by possible relationship which may exist between these different parameters and SOD activity.

Table 1 shows the different values of the correlation coefficient (r) obtained between SOD levels, hemoglobin concentration, the count of reticulocytes, and the count of erythrocytes. Not all correlation coefficient (r) values are statistically significant (p > 0.05).

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 x 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 indicate 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 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 variations indicate that the SOD concentration is not influenced by 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, the count of reticulocytes, and the count of erythrocytes. Not all correlation coefficient (r) values are statistically significant (p > 0.05).

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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 extension 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, splenomegaly myeloid, Vaquez disease, and various visceral cancers has indicated normal SOD activity in each group of patients (7).

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.

REFERENCES

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