Activities of Superoxide Dismutase and Glutathione Peroxidase in Schizophrenic and Manic-Depressive Patients

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Activities of superoxide dismutase (EC 1.15.1.1) and glutathione peroxidase (EC 1.11.1.9) in erythrocytes were evaluated in 50 schizophrenic and 20 manic-depressive patients, who were or were not being treated with different neuroleptic drugs, and results were compared with those for 58 normal individuals. Neuroleptic-treated and untreated schizophrenic patients showed similar activities of superoxide dismutase, about 60% higher than those found in normal individuals ($p < 0.001$). In manic-depressive patients treated with either lithium ($n = 8$) or lithium plus neuroleptic drugs ($n = 12$), superoxide dismutase activities were increased by about 40% over those of normal subjects ($p < 0.001$). Significantly abnormal activities of glutathione peroxidase were found only in the sub-group of schizophrenic women. These results are interpreted in terms of active oxygen species involvement in the psychiatric manifestations.

Additional Keyphrases: superoxide dismutase • glutathione peroxidase • oxygen radicals • mental disorders, neuroleptic drugs

About 30 years ago, Hoffer et al. (1, 2) hypothesized that adrenochrome-linked oxidative stress was a causative factor in schizophrenia. Since then, evidence has accumulated that active oxygen species, generated during auto-oxidation of catecholamines and derivatives, play a role in mental pathology (3, 4). In fact, 6-hydroxydopamine and polyhydroxylated structural analogs are known to produce superoxide anion ($O_2^{-}$), hydroxyl radical ($HO^*$), and hydrogen peroxide ($H_2O_2$) by auto-oxidation (5) and to exhibit neurotoxic activity, perhaps by initiating peroxidative damage to brain tissue (6, 7). Moreover, neuroleptic agents, which are mainly compounds with low oxidation potential (e.g., chlorpromazine), have been demonstrated to inhibit peroxidation of brain lipids (8) and to increase the activity of superoxide dismutase (SOD; EC 1.15.1.1) and of glutathione peroxidase (GSH-Px; EC 1.11.1.9) in rat brain tissues (9). Accumulation of peroxides of serum lipids was reported by Prolipko and Lideman (10) and increased activities of erythrocytic SOD by Michelson et al. (3), although in the latter case the patients studied were ill-defined with regard to medication and other variables. The biochemical bases underlying the presumed triad of oxyradicals, anti-oxidant enzymes, and psychiatric manifestations remain to be firmly established.

To clarify whether medication has any effect on the erythrocytic activities of the anti-oxidant enzymes SOD and GSH-Px, we decided to determine the activities of these enzymes in erythrocytes from schizophrenic patients, untreated or being treated with various neuroleptic drugs, and from manic-depressive patients being treated with lithium or with lithium plus neuroleptic drugs.

Materials and Methods

Patients and samples. Fifty chronic schizophrenic patients—25 women and 25 men, ages 25 to 70 years (mean = 46)—were selected according to criteria of the "International Classification of Diseases" (ICD-9) (11), by psychiatrists from the Hospital Psiquiátrico do Juqueri, São Paulo. All of these patients had been institutionalized for periods ranging from one to 30 years. Twelve of them were being treated with daily oral doses of one or a combination of two or three of the following neuroleptic drugs: chlorpromazine, 125–600 mg; haloperidol, 5–15 mg; promethazine, 50 mg; phe no barbital, 100–200 mg; diazepam, 20 mg; fluphenazine, 100 mg; phenytoin, 100 mg; primadone, 250 mg; carbamazepine, 400 mg; and chlor dia zepoxide, 50 mg. The rest ($n = 38$) received no medication for at least three months before blood sampling.

The manic-depressive patients—18 women and two men, ages 30 to 70 (mean = 46)—were ambulatory patients from the Hospital das Clínicas da Universidade de São Paulo, São Paulo. Twelve of them were taking daily oral doses of Li$_2$CO$_3$ (0.50 to 1.00 g) plus various neuroleptic drugs (chlorpromazine, 100 mg; levomepromazine, 25–100 mg; imipramine, 100–200 mg; or chlorimipramine, 75–250 mg). Eight were taking only Li$_2$CO$_3$.

For comparison, we examined samples from a control group: 56 healthy men and women, ages 18 to 40 years, from a police preparatory school and the Instituto de Química da Universidade de São Paulo (graduate students, faculty members, and employees).

Blood was collected into 5-mL Vacutainer Tubes (Becton Dickinson Co., Rutherford, NJ 07070) containing potassium EDTA. The blood samples were kept on ice, and the corresponding hemolysates for enzymatic assay were prepared on the same day as the venipunctures.

Reagents and apparatus. All reagents were of the highest purity available: glutathione reductase, reduced glutathione, tert-butyl hydroperoxide, NADPH, nitro blue tetrazoli um, riboflavin (all from Sigma Chemical Co., St. Louis, MO 63178); potassium ferricyanide, potassium cyanide, potassium dehydrogen phosphate (all from Merck, Darmstadt, F.R.G.); hemoglobin standard solution (Labtest, Belo Horizonte, MG 30000, Brazil). For spectrophotometry we used Zeiss DMR-10 and DMR-21 instruments (Carl Zeiss do Brasil Ltda., São Paulo, SP 04795, Brazil).

Methods. Within 24 h of preparation of the hemolysate, we assayed SOD and GSH-Px according to Maral et al. (12), using the coupled photoreduced riboflavin/nitro blue tetra-
zolium system for measuring SOD, and the reduced glutathione/NADPH/glutathione reductase system for estimating GSH-Px. SOD and GSH-Px activities were expressed per gram of hemoglobin at 25°C and 37°C, respectively. Expression of the enzyme activities per milliliter of blood gave the same trends when we analyzed the data from the group samples. As controls, we used the standard reaction mixtures for determination of SOD and GSH-Px, containing hemolysate that had been inactivated by boiling for 30 min. Hemoglobin was determined according to Van Kampen and Zijlstra (13). We used Student's t-test for comparison of groups.

Results

Figures 1 and 2 show the distribution of SOD and GSH-Px data, respectively, observed for the sub-groups of schizophrenic and manic-depressive patients as compared with the normal population. Table 1 summarizes the corresponding mean values, standard deviations, and statistical parameters.

For untreated schizophrenic men and women the activities of SOD in erythrocytes were similar to those found in schizophrenic patients treated with neuroleptic drugs (t = 0.06, not sig.). Untreated schizophrenic women showed SOD activities somewhat higher (13%) than those found in untreated patients—the SOD activities were about 60% (50% when expressed per milliliter of blood) higher than in normal individuals (p < 0.001). Manic-depressive patients treated with either lithium or lithium plus neuroleptic drugs also had greater SOD activity than did normal individuals (p < 0.001). The difference between the two groups of manic-depressives was not statistically significant.

The GSH-Px activity data were more scattered than those for SOD. Comparison of the sub-groups of schizophrenic and manic-depressive patients with normal subjects showed statistically significant differences only in the case of untreated schizophrenic women. This group had about a third the GSH-Px activity found in normal individuals (p < 0.01).

Discussion

Studying patients with neuro-psychiatric manifestations, Michelson et al. (3) suggested that increased SOD activities might diminish intracellular concentrations of oxyradicals, affecting the oxyradical-dependent biosynthesis of neurotransmitters and the destruction of endogenous hallucinogens. In the present study we found that, in fact, (a) neuroleptic-treated or not, schizophrenic patients exhibited higher activities of erythrocytic SOD and a trend for lower GSH-Px activities than did normal individuals, and (b) both lithium and lithium/neuroleptic-treated manic-depressive patients had increased activities of SOD and normal activities of GSH-Px in the blood. The lack of any effect of phenothiazine drugs, including chlorpromazine (four patients treated with 2–10 mg/kg body weight per day)—X_sod = 708 (SD = 167) U/g Hb—is in contrast with findings of Roy et al. (9) that there is a dose-dependent effect of chlorpromazine (5–10 mg/kg body weight every two days) on the activity of SOD in the brain tissue of rats. Our results indicate that the increased activities of erythrocytic SOD in these mental disorders may indeed represent a disease-intrinsic peripheral response of the organism to increased superoxide production in the brain.

The trend for decreased erythrocytic activities of GSH-Px reported here for schizophrenics accords with the data of Pecora and Schriftman (cited in 4). Although we cannot explain this result, the implications are potentially serious for these patients: increases in SOD and decreases in GSH-Px might lead to an accumulation of H_2O_2 in their erythrocytes, making them more prone to damage by iron-mediated formation of oxyradicals (14). Brain tissue is reported to be particularly rich in iron compounds (15). Interestingly, the highest activities in erythrocytes and the lowest GSH activities were found in the sub-group of schizophrenic females.
Table 1. Erythrocytic SOD and GSH-Px Activities in the Groups Studied: Statistical Comparison of Data from Patients and Normal Individuals

<table>
<thead>
<tr>
<th></th>
<th>Normal individuals</th>
<th>Schizophrenic patients</th>
<th>Manic-depressive patients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hb, g/L</td>
<td>SOD, U/g Hb</td>
<td>GSH-Px, U/g Hb</td>
</tr>
<tr>
<td>Untreated</td>
<td>144 ± 14 (58)</td>
<td>482 ± 85 (58)</td>
<td>14.9 ± 11.2 (44)</td>
</tr>
<tr>
<td>Treated</td>
<td>133 ± 29 (21)</td>
<td>740 ± 256 (21)</td>
<td>9.8 ± 8.6</td>
</tr>
<tr>
<td>Treated</td>
<td>136 ± 13 (17)</td>
<td>840 ± 136 (17)</td>
<td>5.1 ± 2.6</td>
</tr>
<tr>
<td>Treated Bio</td>
<td>143 ± 20 (12)</td>
<td>772 ± 261 (12)</td>
<td>10.8 ± 7.1</td>
</tr>
</tbody>
</table>

n is the number of individuals in the normal and schizophrenic groups, and the number of analyzed blood samples in the case of manic-depressive patients (20 subjects; see Methods).  
* p < 0.001; b non-significant; * p < 0.01.

(Revised Table 1). The biochemical connection between the psychiatric manifestations and variations in the anti-oxidant enzymes in the blood remains to be elucidated.

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References