Concentrations of Superoxide Dismutase and Superoxide Anion in Blood of Patients with Respiratory Infections and Compromised Immune Systems

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To investigate the involvement of oxygen free radicals and their scavenger systems in the defenses of compromised hosts against pulmonary infections, we determined superoxide anion (SOA) and superoxide dismutase (SOD; EC 1.15.1.1) concentrations in the blood of compromised hosts and noncompromised hosts, with or without pneumonia. In the compromised hosts without pneumonia (compromised controls), SOD concentrations were lower than in noncompromised hosts (healthy controls). However, SOA values in compromised controls did not differ statistically from that in healthy controls. Similar changes were observed in noncompromised hosts with pneumonia. In compromised hosts with pneumonia, SOD concentrations were further decreased by pulmonary infections. By contrast, SOA values were increased in pneumonia. There were, however, no differences in the values for ceruloplasmin among all the groups. The values for $\alpha_2$-macroglobulin and $\alpha_1$-antitrypsin were within normal limits in compromised controls but were greater in compromised hosts with pneumonia. These results suggest that a decreased activity concentration of SOD in compromised controls may be partly responsible for the depression of the host's immune defenses.

Additional Keyphrases: pneumonia \cdot Immune response \cdot acute-phase proteins \cdot enzymic methods

Although considerable progress has recently been made in defining the cellular and molecular mechanisms involved in host defense to infection (1–3), many difficult problems, including infections caused by various opportunistic pathogens, are present in compromised hosts—i.e., patients with altered immunological and other host-defense mechanisms (4–6). Considerable attention has been directed to the possible involvement of oxygen free radical intermediates/scavenger systems in inflammatory processes (1–3), psychiatric diseases (7), Down's syndrome (8), Fanconi's anemia (9), and adult respiratory distress syndrome (10). In particular, the beneficial effects of superoxide dismutase (SOD; EC 1.15.1.1), which acts to decrease the concentration of superoxide anion (SOA) generated by local metabolic disturbances and by chemotactic agents, have been investigated. Little information is available about host-defense mechanisms in compromised hosts.

To estimate how SOD and SOA act on the host-defense system when compromised hosts develop pulmonary infections, we investigated these factors in the blood of compromised hosts with or without pneumonia. We also compared the status of these subjects with regard to other blood factors associated with infections.

Subjects and Methods

Subjects. The study was limited to patients systemically or locally compromised by various factors such as steroid administration and diabetes mellitus (systemic factors); or lung cancer, chronic obstructive pulmonary diseases, or bronchiectasis (local factors in the respiratory tract). Other criteria for selection included: serum creatinine <12 mg/L; creatinine clearance >50 mL/min; leukocyte count >4000/mm$^3$; platelet count >100 000/mm$^3$; no measurable urinary protein (<1 mg/L); and Karnofsky’s score for performance status >50%.

The subjects were divided into seven groups. Group 1 comprised 14 young normal subjects (nine women and five men, mean age 28.0 years). Group 2 was 10 older normal (noncompromised) subjects (four women and six men, mean age 59.9 years). Group 3 consisted of 14 patients (six women and eight men, mean age 60.5 years) with TNM (tumor-lymph-nodes metastasis) stage III or IV lung cancer—compromised hosts. Group 4 consisted of 16 patients (seven women and nine men, mean age 61.8 years) with other underlying diseases such as chronic obstructive pulmonary diseases, bronchial asthma being treated with steroids, bronchiectasis, and diabetes mellitus: other compromised hosts. Group 5 consisted of 13 uncompromised patients (six women and seven men, mean age 57.9 years) with pneumonia. Group 6 was 16 compromised patients (six women and 10 men, mean age 69.0 years) who had lung cancer concomitant with pneumonia. Group 7 consisted of 10 compromised patients (four women and six men, mean age 61.6 years) who also had, simultaneously, other underlying diseases (chronic obstructive pulmonary diseases, bronchiectasis, or diabetes mellitus) and pneumonia. Group 2 was used as the healthy controls; groups 3 and 4 were the compromised controls.

Samples. Venous blood was drawn from normal subjects and patients, early in the morning, under fasting conditions, into 2-mL evacuated glass tubes containing 60 μL of tripotassium EDTA solution (85 g/L). From each specimen, 50-μL aliquots were hemolyzed by mixing with 1.95 mL of cold (4°C) distilled water, the hemoglobin was extracted into 0.5 mL of chloroform/ethanol (3/5, by vol) with vigorous mixing (11). We centrifuged the whole 2.5 mL at 3000 rpm (1400 × g) for 30 min at 4°C and measured SOD activities and SOA concentrations in the resulting clear supernates.

Materials. SOD, xanthine oxidase (EC 1.1.3.22), ferricytochrome c, and fatty-acid-free bovine serum albumin (cat. no. A 7511) were purchased from Sigma Chemical Co., St. Louis, MO. NBT, xanthine, EDTA, sodium carbonate, and CuCl$_2$ were purchased from Nakarai Chemicals Ltd., Kyoto, Japan. Other chemicals were of the highest purity available from commercial sources.

Methods. We measured SOD activity by the method of McCord and Fridovich (12), slightly modified. The reaction...
mixture contained xanthine (100 μmol/L), 150 μg of bovine serum albumin, 25 μmol of nitroblue tetrazolium (NBT) per liter, and 0.1 mL of supernate (or 0.1 mL of distilled water as a blank), brought to a final volume of 3 mL with sodium carbonate buffer (50 mmol/L, pH 10.2) containing 100 μmol of EDTA per liter. After preincubating the reaction mixture for 10 min at 25 °C, we added 30 μg (0.01 U) of xanthine oxidase and let the reaction proceed for 20 min at 25 °C. We terminated the reaction by adding 0.1 mL of a 6 mmol/L solution of CuCl₂. We then measured the increase in absorbance at 560 nm after 20 min, vs a blank. One unit of activity is defined as the amount of enzyme required to inhibit the standard rate of NBT reduction (in the presence of SOD) by 50%.

For the SOA assay, we pre-incubated, for 5 min at 37 °C in a 1.0-cm black cuvette, 0.1 mL of supernate with 1.0 mL of phosphate-buffered saline (phosphate 10 mmol/L, NaCl 150 mmol/L, pH 7.4) containing 2 g of glucose and 2 g of fatty-acid-free bovine serum albumin per liter, with or without 30 μg of SOD, in a slight modification of the method of Johnston et al. (13). To initiate the reaction, we added 0.1 mL of 1.2 mmol/L ferricytochrome c solution. We measured the increase in absorbance at 550 nm in a Model 2400-2 recording spectrophotometer equipped with a thermostated cuvette compartment (Gilford Instrument Labs., Oberlin, OH) and converted the results to nanomoles of reduced ferricytochrome c by using an absorptivity value of 1.96 × 10⁴ L·mol⁻¹·cm⁻¹ (14). We determined the SOA content by calculating the difference between the sample without SOD and the sample with added SOD. Tubes containing only buffer and ferricytochrome c were incubated as above and used as the blank.

We determined the concentrations of α₂-macroglobulin (α₂-MG), α₁-antitrypsin (α₁-AT), ceruloplasmin (Cp), and C-reactive protein (CRP) by using immunodiffusion agar plates obtained from the Medical and Biological Laboratories Corp., Nagoya, Japan (15). Serum samples obtained from venous blood by centrifugation were introduced into agar-containing wells in which each specific antibody to α₂-MG, α₁-AT, Cp, and CRP was incorporated. Diameters of the resulting rings of immune precipitates were measured after 72 h (for α₂-MG), 48 h (for α₁-AT), 48 h (for Cp), and 18 h (for CRP). Standards were included in each run, and each concentration of these factors in sera was estimated from the standard curves.

The protein content of the hemolysate supernate was measured according to the method of Lowry et al. (16), with fatty-acid-free bovine serum albumin as the standard.

**Results**

Table 1 summarizes the concentrations of SOD activities and SOA in whole-blood samples from the various groups. The SOD activity of group 2 (older healthy controls) was slightly lower than that of group 1 (younger healthy controls), but the SOA concentration for group 2 was significantly greater (P < 0.05) than that for group 1. In the compromised controls of group 3 (lung cancer patients) and group 4 (patients with other underlying diseases), SOD activities significantly (respectively P < 0.05 and 0.01) decreased, but SOA concentrations in these groups did not differ from those in age-matched healthy controls (group 2). Similar changes were observed in the uncompromised hosts who had pneumonia (group 5). In the compromised hosts who had pneumonia (groups 6 and 7), SOD activities further decreased, to a greater extent than in the corresponding compromised controls (groups 3 and 4). In addition, SOA values were also higher in groups 6 and 7 than they were in groups 3 and 4. Hematocrits were significantly lower in groups 3, 5, 6, and 7 than in the other groups.

Table 2 lists the leukocyte counts and the concentrations of α₂-MG and other acute-phase reactants in sera obtained from various groups. The leukocyte counts were slightly higher in groups 6 and 7 than those in other groups. However, the SOA values showed a roughly linear increase with these leukocyte counts. There were no significant differences in the concentrations of Cp among all the groups. We noted no definite patterns in α₂-MG values, but α₁-AT values in groups 6 (mean ± SD, 4.575 ± 1.103 g/L) and 7 (3.784 ± 0.546 g/L) increased significantly as compared with those in other groups (range of means, 2.414–3.477 g/L). CRP values were greatly increased in groups with illness also accompanied by pneumonia. Also, in the pneumonia groups both α₁-AT and CRP showed much greater changes than did SOD activities and SOA values but were within normal limits in the compromised controls.

**Discussion**

An organism is compromised by alterations in various host-defense mechanisms (4–6). Defects in each of these host-defense mechanisms increase the risks of infection by

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**Table 1. Activities of SOD and SOA in Whole Blood from Various Groups of Subjects**

<table>
<thead>
<tr>
<th>Groups without pneumonia</th>
<th>Quantity per mg of protein in blood</th>
<th>Hematocrit, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Young controls</td>
<td>14</td>
<td>8.62 ± 0.92°</td>
</tr>
<tr>
<td>G2 Older controls</td>
<td>10</td>
<td>7.53 ± 1.38</td>
</tr>
<tr>
<td>Compromised hosts</td>
<td></td>
<td>5.67 ± 2.14d</td>
</tr>
<tr>
<td>G3 Lung cancer</td>
<td>16</td>
<td>4.54 ± 1.80g</td>
</tr>
<tr>
<td>G4 Others</td>
<td></td>
<td>5.54 ± 1.17f</td>
</tr>
<tr>
<td>With pneumonia</td>
<td></td>
<td>4.11 ± 1.91d</td>
</tr>
<tr>
<td>G5 Noncompromised hosts</td>
<td>13</td>
<td>4.67 ± 1.65g</td>
</tr>
<tr>
<td>G6 Lung cancer</td>
<td>16</td>
<td>0.230 ± 0.053</td>
</tr>
<tr>
<td>G7 Others</td>
<td>10</td>
<td>0.228 ± 0.041</td>
</tr>
</tbody>
</table>

*One unit = amount of SOD that inhibits rate of reduction of NBT by 50%. °Expressed as nanomoles of ferricytochrome c reduced per minute. All values are mean ± SD. d Significantly different from values for older control group at P < 0.05. g Significantly different from values for corresponding compromised hosts without pneumonia (P < 0.05). f Significantly different from the values for corresponding uncompromised hosts with pneumonia (P < 0.02).
specific microorganisms (opportunistic pathogens, as distinct from those that are usually pathogenic anyway) and the risks of other diseases (4–6). In general, compromised hosts may be deficient in two major host-defense mechanisms: nonspecific (whether local or systemic) reactions to the introduction of pathogens into the bodies, and (or) a specific (immunological) host-defense mechanism. Considerable progress has been made in defining both the nonspecific and specific reactions of the host-defense to infection (1–3, 17, 18), but little is known about the relationship between host-defense mechanisms and activated oxygen free radicals or their radical scavengers. To clarify the nonspecific host-defense mechanisms in compromised hosts or during the development of pneumonia in these subjects, in the present study we investigated SOD activity and SOA concentrations as indices of nonspecific reactions in host-defense mechanisms.

Our findings indicate that the activity of intracellular scavengers of oxygen free radicals, especially SOD, was decreased in compromised hosts without pneumonia. Furthermore, despite an increase in SOA values in compromised hosts who also had pneumonia, SOD activity further decreased, resulting from a relative defect of reserved SOD content—that is, an altered intracellular scavenger system in the compromised hosts. In phagocytic cells, intracellular generation of oxygen free radicals (SOA) by NADPH oxidase (EC 1.6.99.6) has been effective as a defense against pathogens, acting to kill them (1, 19, 20). In addition, the intracellular concentration of SOA and, perhaps indirectly, of the hydroxyl radical is controlled by SOD, a metalloenzyme capable of converting SOA and the hydrogen ion to hydrogen peroxide and molecular oxygen (21). Johnston and Lehmeyer (22), however, have shown that neutrophils generate SOA in the extracellular milieu during phagocytosis. Perhaps SOA interacts directly with tissue components by oxidizing or reducing structurally important elements, or through generating even more-reactive intermediates, such as the hydroxyl radical; or perhaps by some as-yet-unknown mechanism it amplifies the inflammatory response by generating additional mediators (23). SOD, the scavenger of the reactive oxygen free radical, is principally intracellular, but it is also present in low concentrations in plasma and other extracellular fluids (24). Reportedly, SOD activity increases in the plasma of patients with various liver disorders (25). In our study, however, SOD activity was difficult to detect in the plasma of various groups (data not shown). Accordingly, we consider that, compared with normal subjects, compromised hosts probably lack sufficient resources within their host-defense network to overcome and remove these oxygen free radicals, so that many types of tissue damage occur, even in an infection-free period. Intracellular and (or) extracellular increases in SOA may have direct cell- and tissue-damaging effects on various vital organs when compromised hosts are further weakened by pneumonia; accordingly, there may be a high concentration of SOA in the inflammatory sites. In the noncompromised hosts without pneumonia, a lack of increase in SOA might be due to scavenger effects of SOD at inflammatory sites. As to whether resources of SOD enzyme or its active accumulation at the inflammatory sites is sufficient, the effects of anemia, reflected in hematocrit, cannot be denied (Table 1).

Another aim of the present study was to investigate whether the measurement of SOD activity in blood would be useful in estimating the host-defense activity, especially in compromised hosts. Although leukocyte counts and acute-phase reactants (α1-AT and CRP) increased during pneumonia, their values were within normal limits in compromised hosts without pneumonia. α2-MG is believed to contribute to the host defenses against invasive pathogens, being the only known inhibitor of the keratinase (EC 3.4.24.10) of Trichophyton mentagrophytes (causal agent of ringworm), the neutral proteinase (EC 3.4.24.4) of Fusiformis nodosus (causing ovine foot-rot), and the collagenase (EC 3.4.24.3) of Clostridium histolyticum (a gangrene organism) in human plasma (26). Measurements of α2-MG did not show a definite trend in compromised hosts with or without pneumonia. Thus determinations of these analytes may not be useful as screening tests when the host-defense activity of compromised hosts without infections must be evaluated. On the other hand, recent reports (27, 28) indicate that Cp, a multifunctional protein in the α2-globulin fraction of human plasma, physiologically may serve as an antioxidant by preventing the accumulation of activated oxygen products, which can initiate lipid peroxidation. In this study, however, we found no significant differences between the serum Cp content of compromised hosts with and without pneumonia, suggesting that Cp also is not a factor suitable for screening the host-defense activity of compromised hosts.

In summary, we postulate that, in addition to such factors as aging, genetics, and the presence of underlying diseases, a low concentration of SOD activity may be crucial in one's
susceptibility to infection by opportunistic pathogens, or in exacerbation of infection. We consider the measurement of SOD in blood to be potentially useful and valuable for estimating the host-defense activity of compromised hosts. However, for a better understanding of the relationship between SOD activity and the host-defense activity of compromised hosts, further studies (e.g., assays of SOD activities and SOA generation in isolated neutrophils and monocytes) should be attempted. These studies are under way in our division.

References