Superoxide Dismutase Activity and Glutathione System in Erythrocytes of Men with Behchets Disease

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Dincer, Y., Alademir, Z., Hamuryudan, V., Fresko, I. and Akcay, T. Superoxide Dismutase Activity and Glutathione System in Erythrocytes of Men with Behchets Disease. Tohoku J. Exp. Med., 2002, 198 (3), 191–195 — In order to clarify whether erythrocyte superoxide dismutase (SOD) activity and glutathione system including reduced glutathione (GSH), glutathione peroxidase (G-Px), glutathione reductase (G-Red), glutathione S-transferase (GST) are impaired in men with Behchets disease (BD) at the first diagnosed time, erythrocyte SOD activity, GSH level, activities of G-Px and G-Red and GST were determined in men with new diagnosed BD. Erythrocyte GSH level, G-Px and G-Red activities were found to be lower, SOD activity was found to be higher in the patients as compared the controls. There was no significant difference between patients and controls for GST activity. Significant positive correlations between GSH and G-Px, GSH and G-Red; significant negative correlations between GSH and SOD, G-Px and SOD, G-Red and SOD were determined. It was concluded that erythrocyte SOD activity and glutathione system are altered in men with new diagnosed BD. It was concluded that these alterations may be a contributory factor for tissue damage associated with BD. Behchets disease; glutathione peroxidase; glutathione reductase; glutathione S-transferase; superoxide dismutase

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Behchets Disease (BD) was first described in 1937 by a Turkish dermatologist, Hulusi Behcet. The disease is characterized by oral and genital ulceration, eye and skin lesions, arthritis and/or arthralgias, thrombophlebitis and diverse visceral and central nervous system involvement (Behcet 1937). Although bacterial or viral infections, genetic, environmental and immunologic factors have been implicated in the pathogenesis of the illness, none of them

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has been proven to be the single cause of BD (O’Duffy 1990). The disease manifests itself symptomatically by tissue injury (Niwa et al. 1982). Investigations have shown that various functions of polymorphonuclear cells (PMNs) in peripheral blood, such as chemotaxis, phagocytosis, lysosomal enzyme activities and reactive oxygen species (ROS) generation are increased in BD (Niwa et al. 1982; Pronai et al. 1991). ROS have been suggested as possible mediators for tissue damage in BD (Tüzün et al. 1998).

Defense against ROS is provided by a system of enzymes and antioxidant compounds capable of preventing excess radical production and neutralising free radicals. Glutathione system including glutathione peroxidase (G-Px), glutathione reductase (G-Red), glutathione S-transferase (GST), and superoxide dismutase (SOD) are major components of intracellular antioxidant defense (Szaleczky et al. 1999).

The purpose of the present study was to examine whether erythrocyte glutathione system and SOD activity are impaired in patients with new diagnose BD.

METHODS

Subjects

We studied 26 men (mean age, 34 ± 10) with complete BD. Since, we have determined a significant difference for GSH level between men and women in healthy individuals recently (Dincer et al. 2002a), we have chosen just men (majority of the applied patients were men) as patient group. Diagnosis of BD were made according to the criteria of the “International Study Group on Behçet’s Disease” (1990). At the time of the study all patients were newly diagnosed and at active stage. Twenty one healthy men (mean age, 31 ± 10) were included as controls. All subjects selected for investigation were non-smokers, non-obese and none of them had been administered antioxidant vitamins and drugs. Declaration of Helsinki was followed in this study. Informed consent was obtained from each subject.

Sample preparation

Blood samples obtained from patients and controls were drawn into heparinized tubes, then centrifuged at 700 × g at 4°C for 15 minutes. Plasma carefully aspirated and packed erythrocytes were washed three times with 0.9% saline solution for removal of the buffy coat. Reduced glutathione (GSH) measurements were performed immediately. Aliquots of the erythrocytes were stored at −70°C for determination the activities of G-Px, G-Red, GST and SOD until analysis.

Biochemical procedure

GSH level in erythrocytes was estimated by the method of Beutler et al. (1963) as spectrophotometrically, using metaphosphoric acid for protein precipitation and 5, 5’ dithiobis 2-nitrobenzoic acid for colour development at 412 nm. GSH levels were expressed as μmol/g Hb. G-Px, G-Red and SOD activity of the erythrocytes were measured by kit from Randox (Catalog no: RS904, GR2368 and SD125, respectively) and expressed as U/g Hb. Erythrocyte GST activity was determined by the Method of Habig et al. (1974). Briefly formation of S-conjugate between GSH and 1-chloro, 2-4 dinitrobenzene was followed by its absorbence at 340 nm. GST activity was expressed as U/g Hb. The Hb concentration was measured by Drabkin’s reagent.

Statistical analysis

The results were expressed as means ± s.d. Statistical significance was ascertained using Student’s t-test. The results were considered significant when the p-value was less than 0.05.

RESULTS

The data are presented in Table 1. Erythrocyte GSH level, G-Px and G-Red activities were found to be lower (p < 0.001 for all) but SOD activity was found to be higher (p < 0.005)
in the patients than those in the controls ($p < 0.001$ for all). There was no significant difference between patients and controls for GST activity. Significant correlations were determined between GSH and G-Px ($r: 0.60$, $p < 0.001$), GSH and G-Red ($r: 0.54$, $p < 0.001$), GSH and SOD ($r: -0.44$, $p < 0.002$), G-Px and SOD ($r: -0.52$, $p < 0.001$), G-Red and SOD ($r: -0.50$, $p < 0.001$).

**DISCUSSION**

It has been suggested that there is a balance between oxidant and antioxidant systems in physiological conditions and, oxidative stress occurs where antioxidant activity reduced, or production of ROS increased (Kalra et al. 1994). Previous studies have demonstrated excessive generation of ROS by stimulated PMNs in BD (Fujita et al. 1984; Pronai et al. 1991). ROS exert their harmful effects by oxidation of lipids, proteins and DNA. It was suggested that increased production of ROS, phagocytosis and chemotaxis of PMNs are responsible for endothelial tissue damage and role of ROS in tissue damage is more remarkable than that of lysosomal enzymes in patients with BD (Niwa et al. 1982).

SOD catalyses the conversion of superoxide anion to H$_2$O$_2$ to O$_2$. Some investigators suggested decreased SOD activity in patients with BD (Doğan et al. 1994; Tüzün et al. 1998). However, on the contrary, Wang et al. (1997) reported elevated SOD activity. In agreement with Wang et al. (1997), increased SOD activity in erythrocytes from patients with BD was determined in the present study. This increase may be a compensatory response to overproduction of superoxide anions in BD. ROS generated by activated phagocytes might also damage the surrounding environment (Kose et al. 2001). Indeed erythrocytes in blood can act as sinks for hydrogen peroxide and superoxide anions generated in plasma or from activated phagocytes: hydrogen peroxide can cross their membranes easily and the erythrocyte has a channel through which superoxide anions can move (Halliwell and Gutteridge 1999a).

In the present study, GSH level was found to be decreased in patients. GSH levels in tissues reflect the dynamic equilibrium between its synthesis and utilization. As far as we know, there is no report indicating alterations in de novo synthesis and/or utilization of GSH in patients with BD. However recently, we have shown in vitro that erythrocyte GSH level decreased when oxidative stress enhanced (Dinçer et al. 2002b, c). Erythrocytes have a plasma membrane rich in polyunsaturated fatty acid chains, thus they are highly susceptible to oxidation, particularly under the inflammatory conditions. Unlike most cells, erythrocytes can not replace oxidized lipids and proteins because of their low metabolic activities (Halliwell and Gutteridge 1999a). Peroxidation of erythrocyte membrane is known to cause impairment of membrane integrity (Knight 1995). GSH can react with ROS itself and it is oxidized (GSSG) at the end of this reaction. GSSG can pass through erythrocyte membrane due to the oxidative stress-induced membrane damage. This mechanism may be responsible for the decreased GSH level in erythrocytes from patients with BD.

On the other hand, proteins are readily targets for ROS. Oxidation of some amino acid residues at active site of an enzyme can lead the inactivation of enzyme (Halliwell and

**Table 1. Determined parameters in the erythrocytes from controls and men with new diagnosed Behcet's disease**

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n: 21</td>
<td>n: 26</td>
</tr>
<tr>
<td>GSH (μmol/g Hb)</td>
<td>4.99±0.92</td>
<td>2.72±0.73*</td>
</tr>
<tr>
<td>G-Px (U/g Hb)</td>
<td>46.71±4.28</td>
<td>33.92±4.21*</td>
</tr>
<tr>
<td>G-Red (U/g Hb)</td>
<td>11.01±1.99</td>
<td>8.66±0.45*</td>
</tr>
<tr>
<td>GST (U/g Hb)</td>
<td>1.42±0.28</td>
<td>1.29±0.36</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>1399±218</td>
<td>1620±276**</td>
</tr>
</tbody>
</table>

*p < 0.001, and p < 0.005 as compared with the control group.
Gutteridge 1999b). G-Px and G-Red may be susceptible to inactivation by ROS via their protein structure. As a matter of fact, G-Px and G-Red inactivation by oxidative stress, in vitro, has already shown by Grzelek et al. (2000) and by us (Dinçer et al. 2002b, c). Decreased erythrocyte activity of G-Px and G-Red in patients with BD, in the present study, may be result of their inactivation by ROS released from activated phagocytes. Dogan et al. (1994) and Kose et al. (1995) reported decreased G-Px activity in PMNs and plasma, respectively, from patients with BD in acute stage, as compared to controls. However, Türtən et al. (1998) reported that there was no significant difference between the patients (both in acute stage and inactive stage) and controls for erythrocyte G-Px activity. On the other hand, G-Px activity depends on the availability of selenium as an essential component; but selenium level has been reported to be lower in patients with BD (Delibas et al. 1991; Kose et al. 1995). This could be also considered as a possible factor involved in decreased G-Px activity in men with BD.

Some reactive metabolites are inactivated by conjugation with GSH catalysed by GST (Wells and Winn 1996). In the present study, although GST activity was slightly lower in the patients, no significant difference was found between groups. As far as we know, there is no report showing G-Red and GST activities in patients with BD.

In conclusion, antioxidant defense including SOD and GSH system may be altered by increased oxidative stress mediated by ROS released from activated phagocytes in men with new diagnosed BD. Recently, impaired antioxidant defense has been implicated as a responsible factor for tissue damage in some diseases such as atherosclerosis (Lynch et al. 1997) and diabetes mellitus (Cakatay et al. 2000). It would be interest to know whether these alterations may be a contributory factor for tissue damage associated with BD. Further investigations are needed to clarify this issue.

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References


