Erythrocyte Catalase Activities in Alcohol Consumption, Medications and Some Diseases

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Catalase (CAT) has a catalytic role in the decomposition of hydrogen peroxide (H$_2$O$_2$) and a peroxidic role in which the peroxide is utilized to oxidize a range of H donors. The objective of the present study was to determine whether alcohol consumption, medications, and diseases have an effect on the erythrocyte on CAT activity or not. For this purpose, the subjects were divided into three different groups with their unique criteria: 1- alcoholic and nonalcoholic, 2- subjects under medication and no medication, and 3- subjects with leukemia, hepatitis, diabetes mellitus, and heart diseases.

CAT activity was measured by the method of Aebi. The hemoglobin levels were determined by Olympus AU-600 auto analyzer. Hematological parameters such as MCH, HCT, MCHC, MCV, and RBC were studied by Coulter STKS instrument. There was no effect of medication on CAT activity 3155±1039 k/g Hb for subjects under medication and 3051±956 for other subjects (p>0.05). When CAT activities of the disease groups with leukemic, cardiac, hepatitis, and diabetic were compared to the control group, there were no significant differences between them. We found that enzyme activity was affected significantly by only alcohol consumption. CAT activities were 3059±958 k/g Hb in nonalcoholic subjects and 3644±984 k/g Hb in alcoholic subjects (p<0.03).

Key words: Catalase - Red Blood Cell - Leukemia - Cardiac Disease – Hepatitis - Diabetes Mellitus

INTRODUCTION

Free oxygen radicals produced by normal aerobic metabolism have been implicated in several pathophysiological mammalian processes: Mammalian erythrocytes have large amounts of CAT, which is a heme-containing enzyme that catalyzes the conversion of hydrogen peroxide to water and oxygen.\(^1\)\(^-\)\(^3\) Catalase (CAT E.C.1.11.1.6) has a dual functional role; a true catalytic role in the decomposition of hydrogen peroxide (H$_2$O$_2$) and a peroxidic role in which the peroxide is utilized to oxidize a range of H donors. It is widely distributed in the body compartments, tissues, and cells. Erythrocytes appear to have high CAT activity compared to other cells because of greatly exposure of erythrocytes to molecular oxygen. Hepatic CAT activities also increase in experimental endotoxemia and hepatitis.\(^4\)\(^,\)\(^5\) In alcoholism, hepatic catalase activities decline, though as aforementioned, it appears to be altered in iron-overload.\(^6\)\(^,\)\(^7\) A wide range of studies support the protective effect of dietary antioxidants for reducing the risk of...
cardiovascular disease. Reactive oxygen species (ROS) appear to be involved in both the development and later complications of diabetes. Moreover, evidence from both animal studies and humans suggest that antioxidant defenses become compromised prior to the development of diabetes. Red cell antioxidant enzymes have been recently studied in malignant lymphomas and results are controversial. The components of the blood antioxidant systems (superoxide dismutase, catalase, ceruloplasmin, glutathione system) take a direct part in molecular mechanisms of the body adaptation under conditions of viral hepatitis infections. Changes in the antioxidant system of red blood cells may be recorded in chronic liver diseases (persistent and active hepatitis, liver cirrhosis). The findings in the literature were the activation of SOD and glutathione reductase; reduction of the activity of total and membrane-bound catalase the content of reduced glutathione. On the other hand, reperfusion of ischemic heart causes the generation of free radicals, and these radicals play an important role in post-ischemic tissue damage. These free radicals are removed by scavenger enzymes and antioxidants in the cell. In a study, it was found that the oxygen free radical reaction in alcohol abusers was pathologically exacerbated and the balance between oxidation and antioxidation was seriously disturbed. Since free radicals and peroxides seem to be involved in the toxicity of alcoholics. Several authors have examined the variations of blood activities of antioxidant enzymes in alcoholics, but published results are somewhat conflicting. Variations of blood antioxidant enzymes observed in patients were of limited amplitude and do not allow the use of either of them as markers of alcohol abuse. Guemouri et al. observed the strong effects in therapy by antidepressants or thyroid hormones. Intake of some drugs (e.g., anti-inflammatory agents, antidepressants, and thyroid hormones) modifies activity of some of the three enzymes (SOD, GPX, and CAT). The behavior of these metabolic parameters reveals the complexity of the diabetic red blood cell metabolism and in addition underlines the fact that the diabetic erythrocytes being less protected from the oxidant agents, has a reduced mean survival as has been evidenced by some authors.

GPX, reduced glutathione (GSH), SOD, and CAT were measured in homogeneous group of patients with untreated hairy cell leukemia and normal controls. GPX, CAT, and SOD activities were significantly lower in patients than in normals. Taken together, these data suggest a decreased activity of red cell antioxidant enzymes in hairy cell leukemia and support a pluripotent stem cell defect of these abnormalities. Therefore, the objective of the present study was to determine whether alcohol consumption, medications, and diseases affect the erythrocyte CAT activity or not.

**MATERIALS AND METHODS**

We prospectively selected patient admitted to Turgut Ozal Medical Center with a clinical diagnosis of leukemia, hepatitis, diabetes mellitus, heart diseases, and other complaints. Subjects were divided into different groups with their unique criteria:

1- With the criteria of alcohol consumption: alcoholic (n=13) and nonalcoholic subjects (n=454)
2- With the criteria of medication: subjects under medication (n=142) and no medication (n=307)
3- With the criteria of disease: leukemia (n=24), hepatitis (n=10), diabetes mellitus (n=18), and heart diseases (n=27)

Venous blood samples from the subjects were taken into heparinized tubes. CAT activity was measured by the reaction of H$_2$O$_2$ decomposing at 240 nm according to the method of Aebi (22). One unit is equal to 1 \( \mu \)mol of H$_2$O$_2$ decomposed/minute. The hemoglobin assay is based on the colorimetric cyanomethemoglobin method and determined by Olympus AU-600 auto analyzer. CAT activity was expressed as k/g Hb. Hematological data that is MCH, HCT, MCHC, MCV, and RBC were studied by Coulter STKS instrument.

Statistical analysis was done by Mann-Whitney U test using SPSS for Windows version 7.5.

**RESULTS**

The results on Table 1 demonstrated that CAT activity of red blood cells from subjects under medication were not significantly different than those of other subjects 7315±1039 k/g Hb for subjects under medication and 3051±956 for other subjects (p>0.05). CAT activities were 3033±982 k/g Hb in healthy subjects, 3432±1126 k/g Hb in
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<table>
<thead>
<tr>
<th>Alcohol Medication Diseases</th>
<th>Healthy</th>
<th>Leukemia</th>
<th>Heart</th>
<th>Liver</th>
<th>DM</th>
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<tbody>
<tr>
<td>CAT (k/g Hb)</td>
<td>3644±985</td>
<td>3059±957</td>
<td>3155±1039</td>
<td>3051±957</td>
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<tr>
<td>RBC (10^6/µL)</td>
<td>9±11</td>
<td>5±34</td>
<td>5±1</td>
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<td>5±1</td>
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<td>MCV (fL)</td>
<td>28±3</td>
<td>29±6</td>
<td>30±8</td>
<td>28±5</td>
<td>29±6</td>
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<tr>
<td>MCHC (g/dL)</td>
<td>33±1</td>
<td>35±17</td>
<td>35±6</td>
<td>35±19</td>
<td>35±20</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>42±5</td>
<td>41±22</td>
<td>42±30</td>
<td>40±17</td>
<td>42±26</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>84±5</td>
<td>87±54</td>
<td>83±63</td>
<td>89±63</td>
<td>89±64</td>
</tr>
</tbody>
</table>

CAT (Catalase), DM (Diabetes Mellitus), RBC (Red Blood Cell), MCV (Mean Corpuscular Volume), MCHC (Mean Corpuscular Hemoglobin Concentration) and HCT (Hematocrit). Data are presented as mean±SD.

leukemia, 2860±596 k/g Hb in heart diseases, 3088±1185 k/g Hb in hepatitis, and 3275±1126 k/g Hb in diabetes mellitus. When the disease groups were compared to the control group, there were no significant differences between them. We found that enzyme activity was affected significantly by only alcohol consumption. CAT activities were 3059±958 k/g Hb in nonalcoholic subjects and 3644±984 k/g Hb in alcoholic subjects (p < 0.03).

**DISCUSSION**

The markers were associated with clinical parameters of the disease indicating that reactive oxygen species could play a role in the development of the pathology. In vitro effects of widely used nonsteroidal antiinflammatory drugs (NSAIDs) and paracetamol were studied on oxidative stress-related parameters of human red blood cells (RBC). Erythrocyte CAT activity was increased by Na-salicylate, acemetacin, and tenoxicam at the therapeutic, and by dipyrone at the high concentration.

Some of our findings are inconsistent with data of other authors. This may be partly explained by large differences in the type and size of the populations studied. Differences in the assay conditions (e.g., types of substrates used and precision of measurements) may also affect the result and the degree of significance.

Biochemical changes induced by alcohol in human organism are concentrating especially on the negative influence on the metabolism of liver. Alcohol-induced oxidative stress is linked to the metabolism of ethanol. Three metabolic pathways of ethanol have been described in the human body so far. More than 90% of ingested ethanol is metabolized in the body to acetaldehyde and acetate. Ethanol is metabolized in the liver via three distinct enzymatic pathways: alcohol dehydrogenase (ADH), the microsomal ethanol oxidizing system (MEOS) and CAT. Each of these pathways could produce free radicals which affect the antioxidant system. Ethanol per se, hyperlactacidemia and elevated NADH increase xanthine oxidase activity, which results in the production of superoxide. Lipid peroxidation and superoxide production correlate with the amount of cytochrome P450 2E1. MEOS aggravates the oxidative stress directly as well as indirectly by impairing the defense systems. Hydroxyethyl radicals are probably involved in the alkylation of hepatic proteins.

The response of the antioxidant defense system in brain subcellular fractions after oral graded doses of ethanol to rat was investigated in a study. Catalase activity was significantly increased in cytosol, synaptosomes and microsomes. In rat thymocytes and cerebellar granule cells, reactive oxygen species (ROS) levels were increased and cell viability was decreased as a result of exposure to ethanol (up to 0.4%). Microsomal P450 and peroxisomal fatty acid oxidation activities were studied in liver of rats after long-term ethanol consumption. Ethanol increased peroxisomal beta-oxidation of palmitoyl CoA and CAT activity in liver. It is widely accepted that alcohol metabolism passes through different mechanisms: alcohol dehydrogenase (ADH) activity in stomach epithelial cells, activity of ADH in the liver, MEOS, hepatocyte CAT activity, and...
nonoxidizing metabolic pathway (production of fatty acid ethylesters).

We observed a significant increase in erythrocyte catalase activities in alcohol users. In the present study, our observations indicated that increased erythrocyte antioxidant enzyme activities were a possible protective mechanism against oxidative stress induced by alcohol. There are several possibilities here: i) Some of the enzymatic reactions in the glycolysis and pentose phosphate pathway can be destroyed by alcohol or products of alcohol, resulting in a lot of changes in erythrocyte metabolism. ii) Alcohol may enhance molecular oxygen toxicity directly or indirectly, thus, CAT activity may be increased as a compensatory mechanism after these changes. iii) CAT activity may be affected by alcohol but we do not know whether alcohol has inhibitor or activator effects on the CAT enzyme activity. iv) All the antioxidant enzymes in the erythrocyte cytoplasm are in a balance. If one of the factors that affect the antioxidant and oxidant status in the cell is decreased or increased, it results in some minor changes. These changes may contain enzymatic or nonenzymatic systems. In addition, our data suggested that CAT activities were not changed in patients with leukemia, cardiac diseases, hepatitis, and diabetes mellitus according to the control groups.

The data in this study indicate that ethanol ingestion may enhance erythrocyte CAT activity. There was a strong relationship between alcohol drinking and the CAT activity. It was concluded that CAT may be used as an important parameter to determine ethanol induced oxidative stress in erythrocytes.

REFERENCES