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A Comparison of Erythrocyte Superoxide Dismutase and Catalase Activity in Patients With Hepatitis C Infection

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Abstract: Hepatitis C virus (HCV) infection remains important due to difficulties in treatment up to a chronic state. It is considered that free radicals, lipid peroxidation and antioxidant defense play a role in various tissue damages, just as in certain types of viral hepatitis. Since only limited data has been reported concerning oxidative stress in viral hepatitis, a comparative study was planned for patients with hepatitis C. In this study, we searched for erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities in patients with HCV infection who had received and not received recombinant interferon alpha. Four groups of patients [Group I: 15 healthy, volunteers served as a control group; Group II: 10 patients with acute HCV infection; Group III: 15 untreated patients with chronic HCV infection; and Group IV: 15 patients who completed six months of interferon therapy (9 million U/week)] were included in the study. In Group I, SOD activity (as means ± standard deviation) was 2213.29 ± 152.01 U/g Hb; in Group II, 2643.03 ± 142.44 U/g Hb; in Group III, 1135.79 ± 122.27 U/g Hb; and in Group IV, 1734.78 ± 183.72 U/g Hb. The difference between the groups was statistically significant ($p < 0.05$). Erythrocyte means ± standard deviation CAT levels in Group I were 252.10 ± 61.09 K/g Hb; in Group II, 253.37 ± 29.68 K/g Hb; in Group III, 291.80 ± 72.54 K/g Hb; and in Group IV 220.43 ± 36.39 K/ g Hb. The difference between Groups I, II and III was not statistically significant ($p > 0.05$), but was for Groups III and IV ($p < 0.005$)

In conclusion, erythrocyte SOD activity increased in acute hepatitis C patients, but decreased in chronic hepatitis C patients, and this decrease was reversed when treated with interferon.

Key Words: Hepatitis C, superoxide dismutase, catalase, interferon alpha

Introduction

The hepatitis C virus (HCV) is widely distributed and causes serious liver disease. Patients with acute hepatitis C (AHCV) leading to chronic state form up to 80% of sufferers and 20% of patients develop cirrhosis that finally may transform into hepatocellular carcinoma (HCC) (1). The mechanisms by which HCV causes cell damage are not well understood. Different mechanisms including immunological liver damage, direct cytotoxicity mediated by different viral products, and induction of oxidative stress have been suggested to play a pathogenic role in this infection (2).

Hydrogen peroxide is not considered as a free radical, but it stimulates damage to the tissue. Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of two superoxide anions (O_2^-) into hydrogen peroxide and molecular oxygen. Superoxide dismutase protects the tissue to a certain degree from the harmful effects of superoxide radicals. There are two forms of SOD, Cu-Zn-SOD and Mn-SOD. Catalase enzyme (CAT) hydrolyzes H_2O_2 into H_2O and 1/2 O_2. The activity of these enzymes plays an important role in the progress of the disease and the care of viral hepatitis C patients.

Since only limited data have been reported concerning oxidative stress in viral hepatitis, a comparative study was performed on patients with hepatitis C (3). In this study, we investigated the level of erythrocyte SOD and CAT as an antioxidant enzyme in acute and chronic hepatitis C infected patients.

Materials and Methods

Four groups of patients [Group I: 15 healthy, voluntary individuals served as control group; Group II: 10 patients with acute HCV infection; Group III: 15

untreated patients with chronic HCV infection; and Group IV: 15 patients who completed six months of recombinant interferon alpha therapy (9 million U/week)] were included in the study. Group I (control group) was selected from non-antioxidant treated, non-smoking and non-alcohol taking healthy subjects. Group II (acute hepatitis C patient group) was selected from patients who had HCV RNA and anti-HCV positive and high ALT-AST level (4-5 times higher than the normal level) and had symptoms like jaundice, malaise or anorexia for 3 weeks. Group III (chronic hepatitis C patients) was selected from patients who were anti-HCV positive for more than 6 months and were showing high levels of ALT and AST. Group IV (chronic hepatitis C patients) was chosen from treated patients who received 9 million U/week recombinant IFN α for 6 months.

Preparation of Erythrocyte Samples

Five milliliters of blood was drawn from the cubital median vein of the subjects into heparinized tubes. The blood samples were centrifuged at 1000 x g for 10 min at 4 °C and the upper phase was taken with a pasteur pipette into an eppendorf tube and stored at –40 °C. The buffy coat on top of the erythrocyte layer was carefully removed and 10 mL isotonic NaCl solution was added. Resuspended erythrocyte was centrifuged at 1000 x g for 10 min and the upper part removed again. Then 10 mL phosphate buffer solution (PBS) was added and the erythrocytes were centrifuged, and the upper buffer part removed by pasteur pipette. The erythrocytes were diluted 10 times with ice cold water, vortexed and stored at –40 °C until used.

Measurement of Catalase Activity

Catalase, (CAT, E. C.I.II. I. 6) enzyme converts H_2O_2, H_2O and 1/2 O_2. Catalase activity was measured by the Aebi method (14). The principle of this method was based on the hydrolyzation of H_2O_2 and decreasing absorbance at 240 nm. The conversion of H_2O_2 into H_2O and 1/2 O_2 in 1 min under standard condition was considered to be the enzyme reaction velocity.

Superoxide Dismutase (SOD) Enzyme Activity Determination

The superoxide dismutase [SOD (E.C.1.15.1.1)] enzyme, which catalyzes the dismutation of the superoxide anion ($O_2^{\cdot -}$) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. SOD activity determination was based on SOD's inhibition of the reaction of superoxide anion (O_2^-), from xanthine by xanthine oxidase and the reduction of nitroblue tetrazolium (NBT) (15).

Determination of Hemoglobin

The colorimetric cyanomethemoglobin procedure was used and determination was performed in an Olympus AU 600 autoanalyzer.

Statistical Analysis

LSD test from the Post Hoc test method and the Kruskall-Wallis test were used for statistical analysis.

Results

The differences between the four groups in superoxide dismutase activity are summarized in the Table. We found that the erythrocyte SOD activity in Group II was 19% higher than in Group I, but in Group III SOD activity was 49% lower than in Group I. SOD activity in Group IV decreased by 22%. These differences were statistically significant ($p < 0.05$).

We also found some differences in CAT activity between the four groups. CAT activity was 13% higher in Group III than in Group I, and this difference was

Table. Levels of SOD and CAT activities in patients with HCV and controls

GROUP NO	SOD (U/gHb)	Activity %	p	CAT (K/gHb)	Activity %	
Group I 15 (control)	2213.29 ± 152.01	0		252.10 ± 61.09	0	
Group II 10	2643.03 ± 142.44	19	$p < 0.05$	253.37 ± 29.68	0.5	$p > 0.05$
Group III 15	1135.79 ± 122.27	-49	$p < 0.05$	291.80 ± 72.54	15	p £ 0.05
Group IV 15	1734.78 ± 183.72	-22	$p < 0.05$	220.43 ± 36.39	-13	$p > 0.05$

Group I: Control; Group II: Acute hepatitis C; Group III: Chronic hepatitis C
Group IV: Hepatitis C patients treated with interferon

significant ($p \leq 0.05$). There was no difference in CAT activity between AHCV patients and the control group ($p > 0.05$). However, CAT activity was 15% lower in Group IV.

Discussion

Recent studies indicate that interferon α (IFN α) and β (IFN β) treatment show beneficial effects in chronic hepatitis C patients (1). When hepatitis C virus infected chronic patients are treated with IFN α, they show a 50% improvement, but when they stop IFN α treatment 50–70% of the patients returned to pretreatment conditions (4–5). This shows that another agent is needed to increase the effectiveness of IFN α (6).

Some studies show that reactive oxygen radicals ($OH^{\cdot}$, $O_2^{\cdot}$ and H_2O_2) increase tissue damage in viral hepatitis patients (7). Reactive oxygen species, including hydroxyl radicals ($^{\cdot}$ OH), superoxide anions ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), lead to the specific oxidation of some enzymes, protein oxidation and degradation (8). Cells are also equipped with enzymatic antioxidant mechanisms that play an important role in the elimination of ROS (9).

Oxidative stress in biological systems can be induced by the depletion of antioxidants and/or by an overload of oxidant species, [i.e., reactive oxygen and nitrogen species (ROS, RNS) and other radicals (R •)], so that antioxidant levels become insufficient (16,17). Sustained oxidative stress damages cellular macromolecules and functions that are maintained and mediated by critical redox systems, thus contributing to the pathophysiology of many diseases (15).

We have studied the level of SOD and CAT enzymes in acute and chronic hepatitis C patients. The level of SOD and CAT enzyme activities were compared with a control group. We found that the erythrocyte SOD activity in Group II was 19% higher than in Group I, but in Group III 49% lower than for Group I. SOD activity in Group IV, increased 27% more than Group III. Differences between the two groups were statistically significant. This result showed that SOD activity was increased in the acute period in order to protect the organism from oxidative damage, while the level of enzymes decreased in the chronic period of the disease. Results of our studies are consistent with reports by Inagaki et al. (18). They showed that SOD activity was decreased in CHC patients.

In another study, reactive oxygen radicals were increased in mononuclear cells (Mn-SOD activity) in chronic hepatitis C patients (19). In a report from Turkey, when patients with chronic active hepatitis C (CAH-C) were compared to controls it was observed that serum thiobarbituric acid reactive substance (TBARS) levels, glutathione peroxidase (GPx) activity and transaminase activities were increased, but total sulfhydryl (t-SH) contents were decreased. Following IFN alpha treatment/three times a week for a period of 6 months, it was observed that elevated TBARS levels and GPx activity were reversed and reduced t-SH contents were increased significantly (20). Studies by Yasuyama et al. (21) showed a decrease of SOD levels in liver tissue in patients with acute and chronic hepatitis accompanied by fatty degeneration compared to patients with inflammatory liver disease of a different etiology.

We found that CAT enzyme activity was approximately at the same level in acute hepatitis C patients and controls, but was 15% higher in CHC patients and 13% lower in interferon treated patients. However, this was not statistically significant. The reason why CAT activity was not increased in acute hepatitis C patients was because of increased reactive oxygen radicals (H_2O_2), and SOD activity was probably the first increasing one. Interestingly, the high activity of Cu, Zn-SOD generates high levels of H_2O_2, which cannot be fully scavenged, because of it not being compensated for by a concomitant increase in CAT activity.

We found a slight increase in CAT activity in the later chronic phase of the disease. The CAT enzyme level fell 13% in IFN α treated CHCV patients.

It was reported that when N-acetyl cystein was used as a glutathione procurso, with IFN α, this treatment increased the level of antioxidant enzymes, but decreased the level of ALT (5). If a patient was infected by the virus, the level of interferon increased in the normal physiologic condition and this stimulates immune (phagocytic activity, cytotoxic activity and natural killer cells) mechanisms in the body (22). Some researchers show that when a patient is infected with a virus, the level of IFN α increased at the beginning but the level of interferon decreased depending on the time period (5). For this reason, when interferon treatment was started in the chronic phase of hepatitis C disease, the response was only 50% (20). It has been suggested that changes in the oxidant-antioxidant balance may play a decisive role in the

progression of liver damage in viral hepatitis and IFN α might be effective in the treatment of liver damage by improving the antioxidant system (22).

In conclusion, oxidative stress can participate in the pathogenesis of HCV infection. The parameters of antioxidant defense may be useful surrogate markers for monitoring hepatitis C infection during hepatoprotective treatment and a combination of interferon treatment with antioxidants may yield better results.

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