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SUPEROXIDE DISMUTASE AND CATALASE ACTIVITIES AND THEIR CORRELATION WITH MALONDIALDEHYDE IN SCHIZOPHRENIC PATIENTS

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ABSTRACT

Free radical mediated pathological processes may have a role in schizophrenia. Free radicals (oxy radicals, such as superoxide, hydroxyl ions and nitric oxide) cause cell injury, when they are generated in excess or when the antioxidant defense is impaired. Both these processes seem to be affected in schizophrenia. In this study we investigated erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities as antioxidant enzymes, malondialdehyde (MDA) as a sign of lipid peroxidation in schizophrenic patients. Activities of superoxide dismutase, catalase and malondialdehyde were greater in patients compared with the control group which may reflect increased oxidative stress in the brain tissue of schizophrenics. In the patient group erythrocyte SOD and CAT activities were weakly negative correlated with MDA concentration. These data reveal that antioxidant defense mechanisms might be impaired in schizophrenic patients. These findings also provide a theoretical basis for the development of novel therapeutic strategies, such as antioxidant supplementation.

KEY WORDS

Lipid peroxidation, Superoxide dismutase, Catalase, Schizophrenia.

INTRODUCTION

There is abundant evidence that free radicals are involved in membrane pathology in the central nervous system and may play a role in neuropsychiatric disorders including schizophrenia (1, 2).

Schizophrenia is a serious hereditary disease. It is a major mental disorder of the brain resulting from abnormalities that arise early in life and disrupt normal development of the brain. The chemical nature of schizophrenic brain is still not completely understood. The brain and nervous system are particularly prone to free radical damage since the membrane lipids are very rich in polyunsaturated fatty acids and areas of human brain are very rich in iron, which plays an essential role in generating free radical species (3, 4, 5).

Free radicals, primarily the reactive oxygen species, superoxide and hydroxyl radicals which are highly reactive having an unpaired electron in an atomic or molecular orbit are generated under physiological conditions during aerobic metabolism. As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids.

The human body has a complex antioxidant defense system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT). These block the initiation of free radical chain reactions (6). The nonenzymatic antioxidant components consist of molecules such as glutathione, alpha-tocopherol, ascorbic acid and beta-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions.

However, when free radicals are generated in excess or when the cellular antioxidant defense

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system is defective, they can stimulate chain reactions by interacting with proteins, lipids and nucleic acids causing cellular dysfunction and even death. Since the chronic schizophrenic patients are under oxidative stress which has exhausted the ability of their antioxidative capacity to adapt to the elevated levels of circulating peroxides, we decided to estimate the level of lipid peroxidation product (MDA) and the activities of antioxidative enzymes superoxide dismutase (SOD) and catalase (CAT) and the correlation of these parameters in schizophrenia.

MATERIALS AND METHODS

This study was performed on 25 patients of age group ranging from 20 to 60 years with chronic schizophrenia. The results were compared with those of 20 age and sex matched normal subjects who comprised the control group. Diagnosis of schizophrenia was made by Diagnostic and Statistical Manual of Mental Disorders (DSM- IV) classification. Subjects who had no other psychiatric disorder and subjects with normal nutritional habits without supplementing any vitamins during the previous 6 months were included. The patients were on treatment for 3-6 months with daily oral doses of one or a combination of two or three of the following neuroleptic drugs: Haloperidol (5-15 mg), Chlorpromazine (100 -400 mg) and Thioridazine (200-400 mg). Informed consent was taken from patients before drawing blood. Venous blood was collected in two tubes with EDTA. In one tube hemoglobin measurement was carried out. The other tube was centrifuged at 3000 g for 10 minutes. Plasma was collected carefully and used for the assay of lipid peroxidation. RBCs were mixed with 0.9% saline and centrifuged. Supernatant was removed. The process was repeated 3 times to prepare RBC suspension, which was used for the assay of SOD and CAT.

Lipid Peroxidation

Plasma MDA level was measured by the modified method of Satoh (7) The assay principle is as follows: Malondialdehyde, a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) in acidic medium to give a pink coloured pigment at 97°C at pH 2-3. The pink color is extracted with butanol and the absorbance read at 535 nm.

In brief, 0.5 ml plasma was precipitated with 10% phosphotungstic acid. The mixture was centrifuged at 3000 g after 10 minutes and the sediment was suspended in 4ml distilled water. To this, 0.5 ml glacial acetic acid and 0.5ml of 0.33% TBA solution were added and the mixture was kept in a water bath at 97°C for 45minutes. 0.05ml of 5M HCl was added after cooling to bring the pH of the solution to<2 [1.6-1.7] (8). Then the pink color was extracted with 4 ml butanol and absorbance read at 535 nm. Values were expressed as nanomoles of MDA formed per deciliter plasma taking the molar absorption coefficient of MDA as 1.5×10^5 (9).

SOD activity

The principle of SOD activity assay was based on the inhibition of nitroblue tetrazolium (NBT) reduction. Illumination of riboflavin in the presence of O_2 and electron donor like methionine generates superoxide anions and this has been used as the basis of assay of SOD. The reduction of NBT by superoxide radicals to blue coloured formazan was followed at 560 nm.

"One unit of SOD activity is defined as that amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions".

The preparation of hemolysate was done by the method of McCord and Fridovich (10). In brief, washed erythrocytes treated with ethanol and chloroform, were centrifuged after 15 minutes, at 4°C and supernatant was used for the assay.

The procedure adopted was that of Beauchamp and Fridovich (11). The reaction mixture contained, 1.9ml of phosphate buffer [pH 7.8], 1×10^{-2} M methionine, 16.8×10^{-5} M NBT and 1.17×10^{-6} M riboflavin, with suitably diluted erythrocyte hemolysate in a total volume of 3ml. Illumination of the solution taken in 10ml beaker was carried out in an aluminium foil lined box, with a 15W fluorescent lamp for 10minutes.Control without the enzyme source was always included. The absorbance was measured at 560 nm. The values were expressed in Units/GmHb (12).

Catalase activity

The catalase activity of the hemolysate was determined by adopting the method of Brannan *et al.* (13). The assay is based on the disappearance of H_2O_2 in the presence of the enzyme source at 26°C. In brief, the hemolysate was prepared from lysed RBC suspension, further diluted by phosphate buffer [pH 7.0]. Here the reaction mixture containing 0.05 M phosphate buffer [pH 7.0] 1.2 mM H_2O_2 and 0.2 ml of diluted hemolysate was allowed to stand for 25 minutes. At the end of which reaction was stopped by the addition of 2.5 ml of peroxidase reagent containing peroxidase and the chromogen system. Peroxidase reduced the H_2O_2 to give a red coloured compound and absorbance measured at 505 nm. With each assay a suitable blank which contained no H_2O_2 and a control which

contained 1ml sodium azide, a catalase inhibitor, were included. Values were expressed as Units/ GmHb (14).

The hemoglobin content of the erythrocytes was determined by the cyanmethemoglobin method (15).

STASTICAL ANALYSIS

The data obtained was analysed using Mann Whitney test. Correlations between the variables were estimated by Pearsons's correlation coefficients.

RESULTS

The plasma MDA levels estimated in schizophrenic patients were significantly high ($p<0.001$) when compared to controls. This indicates lipid peroxidation is significantly increased in schizophrenic patients (Table 1).

The activities of SOD and CAT in erythrocytes were significantly greater ($p<0.047$) and ($p<0.058$) in schizophrenic patients than in controls (Table 1).

Erythrocyte SOD activity and CAT activity were weakly negative correlated with MDA concentration in the patient group and a strong negative correlation of MDA concentration with SOD and CAT activities was observed in the control group (Fig. 1).

DISCUSSION

The results indicate that there is increase in free radical generation in schizophrenia and antioxidant defense is impaired. The free radicals play an important role in the genesis of structural and functional changes of neuronal membrane that could be responsible for the beginning or aggravation of the basic disease (16,17, 18). The brain and nervous system possess high potentials for the initiation of free radical reactions, which relative to other tissues, can cause more damage in the brain and nervous system due to insufficient antioxidant protection and existing intensive aerobic metabolism accompanies with oxygen radical production (19). There are several ways by which excess free radicals may be generated in the brain. The metabolism of catecholamines, such as dopamine and norepinephrine is probably associated with free radical formation and conditions associated with increased catecholamine metabolism may increase the free radical burden. Antipsychotic drugs also can cause an increase in the metabolic turnover of catecholamines(20). Of the different brain regions the basal ganglia may be particularly at risk for radical induced damage because they contain large amounts of iron (which can be associated with increased free radical production through the Fenton reaction).

In this study mean plasma MDA level was significantly higher in patients group than in the control group. This may show the presence of increased oxidative stress. We have also found increased erythrocyte SOD and CAT activities in patients with schizophrenia. It is likely that sustained oxidative stress may increase SOD and CAT activity. Decreased antioxidant defense probably exist later in patients under chronic treatment with neuroleptics (21, 22). The identified

Table 1. Comparison of MDA, SOD activity and catalase activity in schizophrenic patients and controls

Subjects	MDA nmol/dl Mean ± SD	SOD units/gm Hb Mean ± SD	Catalase units /gm Hb Mean ± SD
Controls N = 20	180.45 ±53.25 (41.0 - 261.5)	4561.12 ±2478.30 (1302.12 -8578.15)	6244.21 ± 2138.76 (2400.0 -10120.0)
Schizophrenic patients N=25	322.20 ±166.65 (117.9 -923.0)	6298.07 ± 2300.64 (2757.8 - 10923.46)	7379.97 ±1949.30 (5000- 14727)
p value	0.001	0.047	0.058

N = Number of subjects.

SD = Standard deviation.

p value calculated by Mann - Whitney test.

The figures in the parentheses indicate the range.

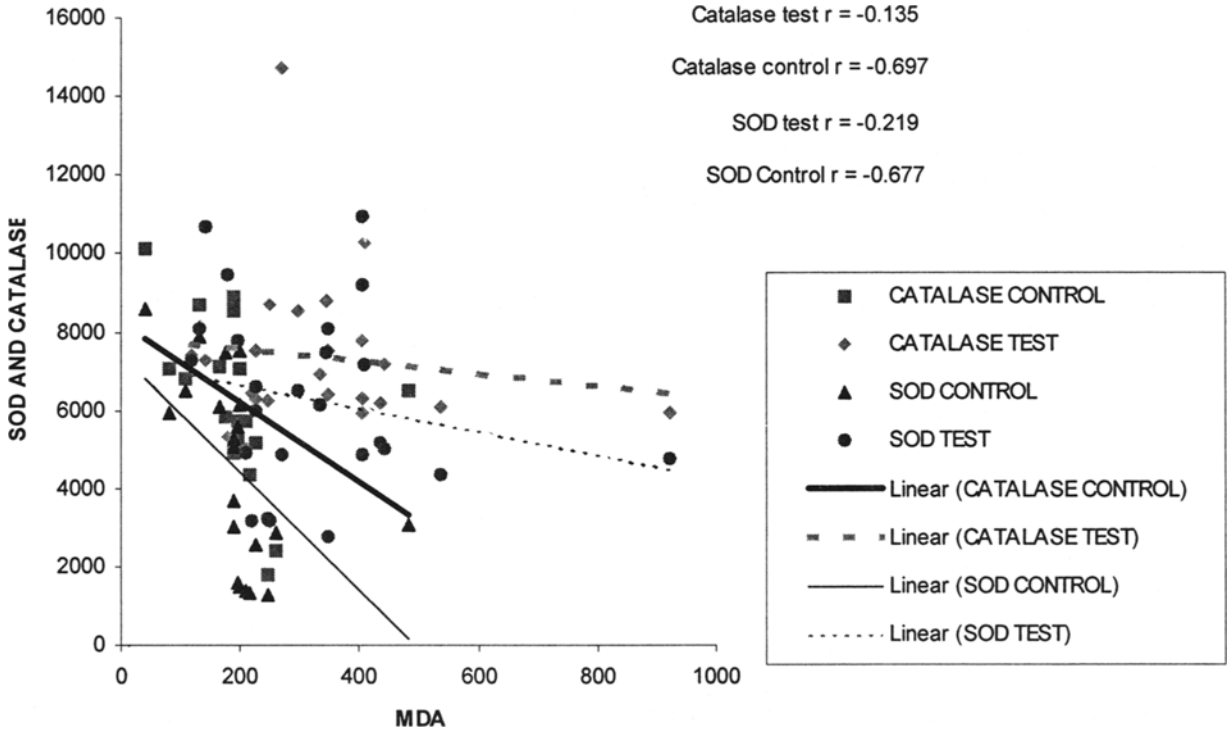

Fig. 1. Correlations of SOD, catalase with MDA

increase in SOD and CAT activity could be an adaptive response of these enzymes to increased production of oxygen following oxidative decomposition of catecholamines. The changes in activities of antioxidant enzymes might offer some important clues to explain pathologic mechanism of abnormal free radical metabolism. The molecular mechanisms of oxyradical mediated cellular pathogenesis are well understood. OH · and HO2· radicals are involved in cell damage by their actions on phospholipids, proteins and nucleic acids. The reaction products of these molecules have been used as indices of oxyradical injury. The membrane phospholipids, specifically esterified polyunsaturated fatty acids are converted by peroxidation to MDA, which can be analysed by reactivity to thiobarbituric acid. Increased levels of thiobarbituric acid reaction products have been found in the cerebrospinal fluid of neuroleptic treated patients (23,24) and also in plasma of schizophrenic patients with or without tardive dyskinesia (25).

The facts that mean erythrocyte SOD activity was higher in patient group than in controls may be indicative of increased superoxide generation by whichever mechanism like increased catecholamine metabolism or antipsychotic drugs. The free radicals produced during the metabolism of catecholamines may result in neurotransmission abnormalities at dopamine terminals. The brain has certain attributes that make it exceptionally vulnerable to free radical attack. It has highly oxygenated structures responsible for almost one-fifth of the bodies total oxygen. In addition, there is disruption of brain energy metabolism mediated by antioxidant perturbation (26, 27, 28). The weakly negative correlation between erythrocyte SOD activity and CAT activity and MDA may show that duration and severity of the disease might parallel increased oxidative stress. Intense oxidative stress and decreased antioxidants may contribute to neuronal death and alter the information processing in schizophrenia. We need more studies to use antioxidants such as vitamin C and E in addition to current drug therapy in schizophrenia.

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