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ORIGINAL PAPER

Alterations in Antioxidant Enzymes During Aging in Humans

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Abstract The oxidative stress theory of aging offers the best mechanistic elucidation of the aging phenomenon and other age-related diseases. The susceptibility of an individual depends on the antioxidant status of the body. In humans, the antioxidant system includes a number of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), nonenzymatic antioxidants such as glutathione (GSH), protein –SH, ascorbic acid, and uric acid, and dietary antioxidants. Antioxidant enzymes form the first line of defense against reactive oxygen species. In an earlier report, we showed that the plasma antioxidant potential in humans decreases as a function of age and that there are compensatory mechanisms operating in the body which are induced to maintain the antioxidant capacity during aging. In the present study, we report the relationship between human aging and antioxidant enzymes SOD and CAT; we also correlate the activity of these enzymes with the antioxidant capacity of the plasma. Our results show a significantly higher plasma SOD and CAT activity in older individuals than in younger individuals. The induction in activity of SOD and CAT during human aging may be a compensatory response of the individual to an increased oxidative stress.

Keywords Human aging · Oxidative stress · Superoxide dismutase · Catalase

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Introduction

Aging is a multifactorial process involving morphological and biochemical changes in single cell and in the whole organism. The exact mechanism underlying aging is not well understood, but there is enough evidence to suggest a possible relationship between life span and production of free radicals. It has been suggested that aging could be caused by the accumulation of deleterious effects of reactive oxygen species (ROS), throughout the life [1]. Aerobic cells produce ROS as a byproduct of their metabolic processes. The ROS cause on oxidative damage to macromolecules under conditions in which the antioxidant defense of the body is overwhelmed [2]. A certain amount of oxidative damage takes place even under normal conditions; however, the rate of this damage increases during the aging process as the efficiency of antioxidative and repair mechanisms decrease [3, 4].

The correlation between antioxidant capacity and oxidative damage during aging has been reported in several tissues in different species [3, 5, 6]; however, data on changes of oxidative stress markers in plasma and erythrocytes of healthy populations during aging are few and sometimes contradictory [7–9]. Recently, we have reported a significant age-dependent decline in plasma antioxidant capacity, measured in terms of Ferric Reducing Ability of the Plasma (FRAP) values [10]. Since antioxidant capacity of the plasma is related to dietary intake of antioxidants [11], it is important to study the correlation between the antioxidant capacity of the plasma and markers of oxidative stress in different populations.

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide radical ($\cdot O_2$) to hydrogen peroxide (H_2O_2). Although H_2O_2 is not a radical, it is rapidly converted by fenton reaction into ·OH radical which is very reactive.

Catalase (CAT) metabolizes H_2O_2 into H_2O. Oxidative stress is the primary factor regulating the gene expression of these enzymes [12]; however, there are many other factors, such as inflammation, hormonal regulation, and aging [13] that also influence the activity of antioxidant enzymes. In the present study, we report the age-dependent alteration in the activity of plasma SOD and CAT in the Indian population. We also correlate these parameters with total plasma antioxidant potential measured in terms of FRAP [10].

Material and Methods

The study was carried out on 80 normal healthy subjects of both sexes between the ages of 18 and 85 years. The subjects were screened for diabetes mellitus, asthma, tuberculosis, or any other major illness. None of the subjects were smokers or were taking any medication. All persons gave their informed consent for the use of their blood samples for the study. The protocol of study was in conformity with the guidelines of the Institutional Ethical Committee.

Superoxide Dismutase (SOD) Assay

Human plasma SOD activity was determined by a modification of the method described by Marklund and Marklund [14]. Plasma (50 µl) was incubated with 2.85 ml of 0.05 M Tris–succinate buffer (pH 8.2) at 37°C for 20 min. Reaction was started by adding 100 µl of 8 mM pyrogallol. The increase in absorbance was recorded at 412 nm for 3 min at 30-s intervals. An appropriate blank was also run simultaneously. The SOD activity is expressed in Unit (50% inhibition of pyrogallol autoxidation per minute) per milligram protein.

Catalase (CAT) Assay

Plasma CAT activity was determined by a modification of the Beers and Sizer method [15]. The final assay mixture incubated at 37°C contained 3 ml of 50 mM phosphate buffer (pH 7.0) containing 30 mM H_2O_2, reaction was started by adding 25 µl plasma. The decrease in absorbance was recorded at 240 nm for 3 min at 30-s intervals. The CAT activity is expressed as µmol H_2O_2 decomposed min^{-1} mg^{-1} protein.

Plasma protein was estimated following the method of Lowry et al. [16].

Statistical analyses were performed using the software PRISM 4. Relationships between various parameters were assessed using Pearson correlation coefficient (r).

Results and Discussion

Detrimental effects caused by ROS occur as a consequence of an imbalance between the formation and inactivation of these species. Inactivation and removal of ROS depend on reactions involving the antioxidative defense system. The capacity of antioxidant defense is determined by the contributions of certain vitamins (A, E, and C), β carotene, reduced glutathione, and antioxidative enzymes. Wide inter-individual variations may exist regarding antioxidative capacity, thus affecting individual susceptibility against deleterious oxidative reactions. However, very limited information exists concerning the biological variation of antioxidative enzymes in representative population samples. Changes in oxidative damage and antioxidant capacity during aging have been shown in several tissues in different species [3, 5, 6, 17].

Figure 1a shows the age-dependent increase in the activity of plasma SOD activity in humans. We observe a significant ($P < 0.0001$) positive correlation (r = 0.7210) between plasma SOD and human age. To analyze the

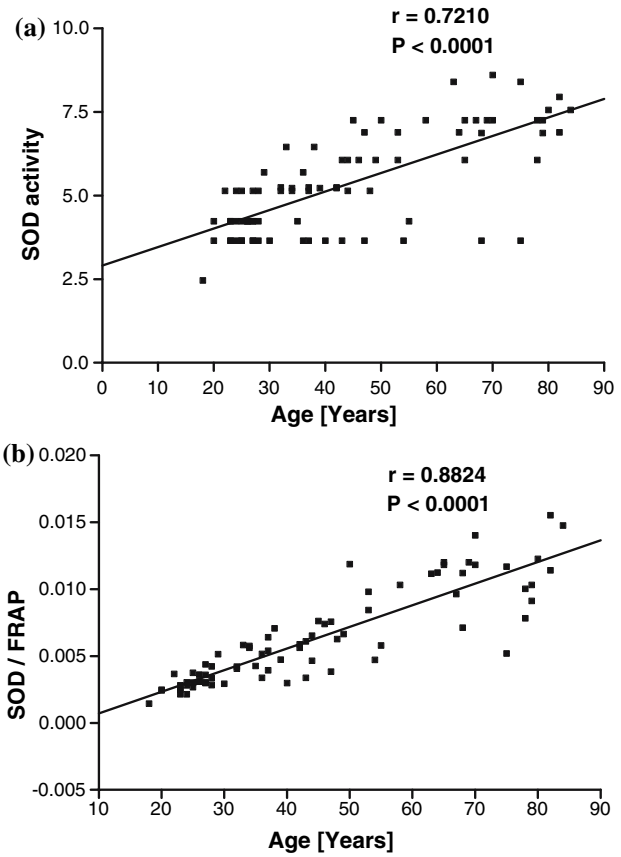

Fig. 1 (**a**) Activity of plasma superoxide dismutase (SOD) plotted as a function of human age (**b**) Plot of quotient: SOD activity/FRAP values as a function of human age. FRAP values expressed as µ mol Fe (II) per liter of plasma from Rizvi et al. [10]

correlation of plasma SOD activity with plasma antioxidant capacity, measured in terms of FRAP values [10], we plot a quotient: SOD activity/FRAP as a function of human age (Fig. 1b). The plot shows a strong positive correlation (r = 0.8824) between SOD and total plasma antioxidant potential. Our results thus confirm that human aging is associated with induction in the activity of SOD, which correlates with decrease in antioxidant potential. The increased activity of SOD could be, due to a compensatory mechanism in response to increased oxidative stress during the aging process in humans. Significantly, there is a great variability in the literature regarding SOD activity during aging that is hard to explain [3, 18–20].

Figure 2a shows the age-dependent increase in the activity of plasma catalase activity in humans. We observed a significant ($P < 0.0001$) positive correlation (r = 0.6151) between plasma catalase and human age. Figure 2b is a plot of quotient: catalase activity/FRAP as a function of human age. The plot shows a strong positive correlation (r = 0.8542) between catalase activity and total plasma antioxidant potential.

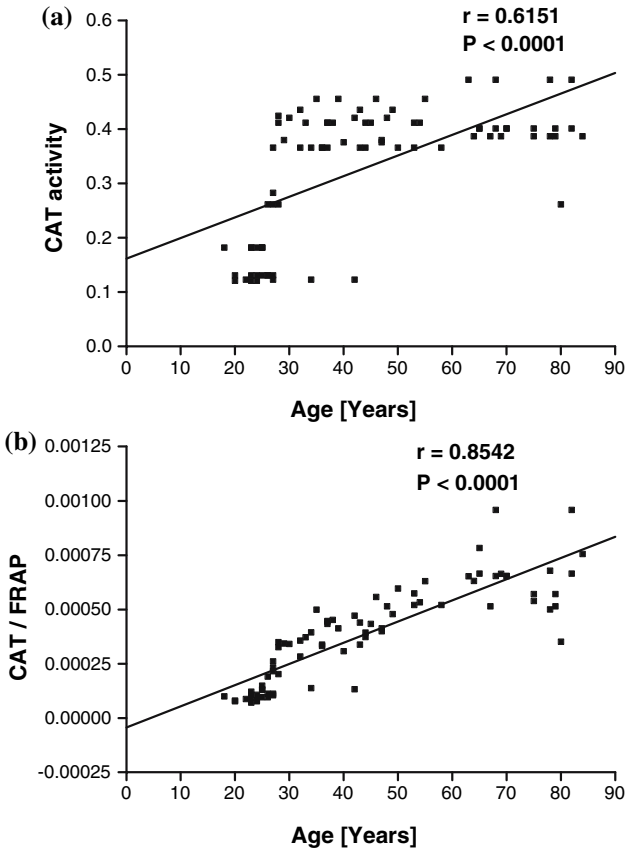

Fig. 2 (**a**) Activity of plasma catalase (CAT) plotted as a function of human age (**b**) Plot of quotient: CAT activity/FRAP values as a function of human age. FRAP values expressed as μ mol Fe (II) per liter of plasma from Rizvi et al. [10]

Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and the hydroxyl radical are known to play a role in organ damage associated with aging. Aged persons exhibit higher production of ROS than young and middle-aged persons. It has been suggested by many authors that oxidative stress is a possible cause for aging.

Among various antioxidant mechanisms in the body, SOD is thought to be one of the major enzymes that protects cells from ROS. There is a suggestion that the activity of antioxidant enzymes may play an important role in determining the life span of animal species [21].

The increased activity of antioxidant enzymes among aged persons seems to be a compensatory mechanism against high levels of ROS in old age; we hypothesize that this adaptability ensures that oxidation-mediated damage takes place at a rate that determines the pace at which we age, defined by Harman as the 'inborn aging process' [22]. Our recent report of an increase in the activity of erythrocyte membrane plasma membrane redox system [10] also corroborates our present finding of an increase in the activity of plasma SOD and CAT. We explained the higher PMRS activity, as a protective mechanism of the system for efficient extracellular dehydroascorbic acid reduction and ascorbate recycling under the condition of oxidative stress. Looking at both of the findings (higher erythrocyte PMRS and increased activity of plasma SOD and CAT) in conjunction, we hypothesize that the human body has inherent compensatory mechanisms against oxidative stress; however, this capacity is overwhelmed during aging. Our findings also emphasize the need to establish age-dependent reference values for SOD and CAT involving their role in different disease conditions.

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