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ALTERATIONS OF ANTIOXIDANT ENZYME LEVELS AND OXIDATIVE STRESS MARKER IN LIVER AND SKELETAL MUSCLE DURING AGING.

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ABSTRACT The aging process has been described by various theories, but, the free radical theory of aging has received widespread attention. The present study was carried out to study the influence of aging on antioxidant enzymes; Superoxide Dismutase (SOD), Catalase (CAT) and oxidative stress marker expressed as thiobarbituric acid reactive substances (TBARS;MDA) in the liver and skeletal muscle (Gastrocnemius) of Swiss albino mice at different age intervals (0 day-6 months). The antioxidant enzyme levels were decreased in moderately aged mice when compared to young mice in both tissues. In liver, no definite trend in the levels of MDA was observed however in skeletal muscle an age dependent decrease was observed in early stages. Our study suggests that free radicals could be the causative agents of aging process in which antioxidant enzymes have a definite regulatory contribution.

KEYWORDS : Reactive Oxygen Species, free radicals, aging, oxidative stress.

1. Introduction

In Recent decades the study of aging has expanded both in depth and breadth. Aging is a multifactorial process involving morphological and biochemical changes in a single cell and in the whole organism. The changes that occur during aging may be attributed to environmental factors, inborn processes or underlying disease (Harman, 1993). The exact mechanism underlying the aging process 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), through out life (Harman, 1956). Aerobic cells produce ROS which mainly includes superoxide anion(O_2^-), hydroxyl radical(OH^-) and hydrogen peroxide(H_2O_2) as a byproduct of their metabolic processes. The ROS cause an oxidative damage to macromolecules under conditions in which the antioxidant defense of the body is overwhelmed (Droge, 2002). 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 decreases (Inal et a., 2001; Gil et al., 2006)

The production of ROS is mostly dependent on the intrinsic metabolic rate and oxygen consumption. Since we live in an oxygenated environment, our cells have developed an impressive repertoire of strategies to detect and detoxify metabolites of molecular oxygen that could impair the organism's survival. Antioxidant enzymes are universally expressed across all species to protect cells from oxidative stress. Superoxide Dismutase (SOD) (EC 1.15.1.1) is an enzyme that speeds the conversion of superoxide anion radical (O_2^-) to hydrogen peroxide, while catalase (CAT) (EC 1.11.1.6) converts hydrogen peroxide in to water (Beckman and Ames, 1998). A delicate equilibrium between ROS production and antioxidant defenses determines the degree of intracellular oxidative stress. Increased oxidative stress has been hypothesized to play an important role in the aging process by disrupting cellular homeostasis (Biesalski, 2002).

Therefore, the present study was aimed to analyze the activities of two protective enzymes, SOD and CAT and the levels of malondialdehyde (MDA).

2. Materials and Methods

Reagents: All reagents used were of Analytical Grade (AR) and purchased from Sigma Chem. Co. (St. Louis) and Merck Darmstadt (Germany).

2.1 Animals and Experimental Protocol: Swiss albino mice (Lakka strain) of different age group (0 day (birth), 1 day, 1 week, 1month, 3 month, 6 months) used in this study were procured from Haryana Agricultural University, Hisar. The entire animal care and different experimental procedures were approved by the Institutional Animal Ethics Committee, CSK HPKV, Palampur. The mice were housed in clean polypropylene cages and maintained under temperature controlled room (25±2^{0}C) with a photoperiod of 12 hrs light and 12 hrs dark cycle. The animals were fed with standard mice diet and water *ad libitum*. Six animals of each group showing no sign of morbidity were sacrificed for assaying the activities of antioxidant enzymes and lipid peroxidation. The animals were sacrificed by cervical dislocation. Liver and skeletal muscle (Gastrocnemius- both red and white) fibers were dissected from surrounding tissue on a dissection board and rinsed with ice cold 0.9% w/v NaCl to remove blood. The tissue samples were placed in phosphate buffer solution (pH 7.0) and maintained under cold conditions (4^{0}C). The tissue samples were homogenized in prechilled pestle and mortar with phosphate buffer saline (pH 7.0). After centrifuging at 15000 rpm and 4^{0}C for 30 minutes, the supernatants were removed and used for enzyme analysis and protein determination.

2.2 Evaluation of antioxidant enzyme activities The activity of superoxide dismutase (SOD) was estimated by spectrophotometric method as described by Winterbourne et al., 1975 and Marklund and Marklund (1974). The activity of Catalase (CAT) was measured according to the methods described by Aebi (1983) and Beers and Sizer (1952). Lipid peroxidation (LPO) was determined in terms of MDA (malondialdehyde) production, as described by Buege and Aust 1978 and Shafiq-ul-Rehman (1984).

2.3 Determination of Protein: Protein contents in the homogenate were estimated by using Coomassie Brilliant Blue (CBB G-250) dye as reagent. The standard graph for protein was prepared by using Bovine serum albumin as standard. The absorbance of blue color developed was measured at 595nm(Bradford 1974).

2.4 Statistical Analysis

Statistical analysis of the data was carried out by one way analysis of variance (ANOVA) followed by Dunnet's test using Graph Pad Instat version 3.00 for windows. P value ≤0.05 was considered as significant. All the values are expressed as mean ± SE.

3. Results

The mean ± SEM values of SOD, CAT and TBARS (MDA) at different age intervals in liver and skeletal muscle are summarized in table 1 and table 2 respectively. In the present study, an age related increase in SOD activity was observed in both liver and muscles from the age 0 day to 3 months followed by a decrease in the levels at the age of 6 months. Similar trend has been observed for the CAT level in both tissues from the age 0 day to 3 months followed by a decrease in the levels of enzymes at the age of 6 months. No definite trend in the levels of MDA was noticed with aging in liver. However, in skeletal muscle an age dependent decrease in the levels of MDA at the early stages of aging (1 day -3 months) was observed.

Table 1: Changes in levels of antioxidant enzymes (SOD, CAT) and levels of MDA at different stages of aging in liver of mice.

Age Parameters	0 Day	1 Day	1 Week	1 Month	3 Month	6 Month
SOD (Units/ mg protein)	1.422± 0.0159*	1.591 ± 0.0107*	1.830 ± 0.0066*	2.561± 0.0121*	3.191 ± 0.0083*	2.791± 0.0127*
CAT (µM of H2O2 decomp /min/ mg protein)	1.860 ± 0.0149*	2.081± 0.0367*	2.252 ± 0.0125*	2.842± 0.0175*	3.641 ± 0.0115*	3.290 ± 0.0118*
TBARS (LPO) (nM MDA /mg protein)	2.601 ± 0.0142*	2.901 ± 0.0826*	2.340 ± 0.0380*	3.511 ± 0.0125*	4.441 ± 0.0081*	3.811± 0.0082*

All values are Mean ± SE (n=6)
The superscript * represents level of significance at P≤0.05

Table 2: Changes in levels of antioxidant enzymes (SOD, CAT) and levels of MDA at different stages of aging in skeletal (gastrocnemius) muscle of mice.

Age Parameters	0 Day	1 Day	1Week	1Month	3 Month	6 Month
SOD (Units/ mg protein)	2.221 ± 0.0002*	2.650 ± 0.0012*	3.192± 0.0083*	3.451± 0.0026*	3.991 ± 0.0088*	2.920 ± 0.0042*
CAT (µM of H_2O_2 decomp /min/ mg protein)	2.451 ± 0.0004*	2.570± 0.0056*	3.280 ± 0.0009*	3.761± 0.0005*	4.322 ± 0.0005*	3.161± 0.0004*
TBARS (LPO) (nM MDA/ mg protein)	2.620 ± 0.0004*	4.031 ± 0.0002*	3.830 ± 0.0004*	3.332± 0.0003*	2.921 ± 0.0003*	4.460± 0.0003*

All values are Mean ± SE (n=6)
The superscript * represents level of significance at P≤0.05

4. Discussion

Among various antioxidative mechanism in the body, SOD is thought to be one of the major enzyme which protects against tissue damage caused by the potentially cytotoxic reactivities of radicals(Carillo et al., 1992; McCord and Fridovich, 1969) In the present study, an increase in the SOD activity (0 day-3 months) in both tissues may be due to more aerobic environment because in order to be prepared for the relatively enriched O_2 world after birth, a prenatal increase in Antioxidant Enzyme (AOE) activity is necessary. When the cells are becoming more and more aerobic, the enzyme is subjected to increase turnover in an attempt to overcome the deleterious effects of toxic reactants. Production and activity of AOE increases markedly in the final days before birth, and even more so after birth (Frank and Sosenko, 1987). Decrease in the SOD activity at the age of 6 months indicates either reduced synthesis of enzymes or elevated degradation or inactivation of enzyme as age advances(Alper et al., 1998).The enzyme activity of CAT also showed similar trend as that of SOD enzyme during the early stages of aging i.e. it increases from the age of 0 day to 3 months. The increase in the activity is explained on the basis that when the activity of SOD enzyme increases, the product of its dismutation reaction, H_2O_2 which is the substrate for CAT enzyme also increases. Catalase enzyme needs high H_2O_2 concentration to function effectively as it has low affinity for H_2O_2 (Halliwell and Gutteridge, 1999). The decreased CAT activity at the age of 6 months may be because of high reactive oxygen metabolites production especially O_2^- and H_2O_2 during aging process and cause oxidative stress to the tissues (Kono and Fridovich, 1982).

The levels of MDA showed no definite trend with aging in liver of mice. An increase in the levels of MDA at the age of 0 and 1 day may be explained on the basis that there is sharp transition from the essentially glycolytic state of neonate to a high reliance on oxidative phosphorylation for supplying energy demands in the first few weeks after birth. There is an increase in the number and volume of mitochondria. As the number of mitochondria increases, the rate of ROS production also increases resulting in an increase in the levels of MDA (Morten et al., 2006).

The decrease in the level at the age of 1 week may be attributed to the presence of sufficient antioxidant enzymes to scavenge free radicals. The reported increase at the age of 1 and 3 months is explained on the basis that the levels of AOE were not sufficient enough to detoxify the free radicals produced in liver, also when the cells or tissues are in stressful conditions, there is an increase in the ROS production Beckman and Ames, 2000; Chance et al., 1979). The reduction of lipid peroxidation at 6 months related to decrease in the levels of enzyme at this stage is difficult to explain but the role of other antioxidant enzymes particularly GSH-Px, GST participating in the disposal of ROS is not ruled out.

In skeletal muscle the reduction observed in the levels of MDA in the early stages (0day-3 months) may be related to the increase of SOD and CAT activities in this tissue at these stages which helps in detoxification of free radicals. The reported increase at the age of 6 months showed that with aging the level of AOE starts declining and their levels are not sufficient to detoxify the free radicals. Ji *et al* (1990) reported that lipid peroxidation was increased in skeletal muscle homogenate of aged rats.

Looking at these findings, increased activity of SOD and CAT in conjunction, we hypothesize that the animal (mammalian) body has inherent compensatory mechanisms against oxidative stress; however, this capacity is overwhelmed during aging. In conclusion, our findings suggest that free radicals could be the causative agents of aging process in which antioxidant enzymes have a definite regulatory mechanism. However, further detailed studies involving more antioxidant enzymes are needed to further clarify cellular mechanisms involved Inliverandmuscle.

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