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Oxidative stress-mediated macromolecular damage and dwindle in antioxidant status in aged rat brain regions: Role of L-carnitine and DL-α-lipoic acid

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Abstract

Background: The free radical theory of aging has significant relevance in a number of age-related neurological disorders. Too many free radicals create cellular pollution that shuts down energy levels. They have also been implicated in the loss of physiological functioning associated with the aging of post mitotic cells such as the brain. The activities of enzymatic antioxidative defenses decrease in rat brain may be possible causes of age-associated increase in oxidative damage to macromolecules.
Methods: We determined whether DL-α-lipoic acid (100 mg/kg body weight/day), and L-carnitine (300 mg/kg body weight/day) together when administered for 30 days declines the rate of oxidative stress-mediated macromolecular damages such as lipid peroxidation (LPO), protein carbonyl (PCO) and DNA protein cross-links in different anatomic regions (cortex, striatum and hippocampus). The activities of antioxidant enzymes in programmed aging were evaluated in the cortex, striatum and hippocampus of young and aged rat brain regions.
Results: Aged rats elicited a significant decline in the antioxidant status and increase in LPO, PCO and DNA protein cross-links as compared to young rats in all the 3 brain regions. The increase in LPO, PCO and DNA protein cross-links were (35.8%, 35.6%, 43.5%) in cortex, (32.5%, 40.3%, 29.8%) in striatum and (62.7%, 42.4%, 34.9%) in hippocampus, respectively, in aged rats as compared to young rats. Co-supplementation of carnitine and lipoic acid was found to be effective in reducing brain regional LPO, PCO and DNA protein cross-links and in increasing the activities of enzymatic antioxidants in aged rats to near normalcy.
Conclusion: The combination of L-carnitine and lipoic acid overcame the oxidative stress induced rate of lipid peroxidation, protein carbonyl formation, accumulation of DNA protein cross-links and deficits in antioxidant enzyme activities in various brain regions of aged rats.

Keywords: Aging; Free radicals; Macromolecular damage; Antioxidant enzymes; L-carnitine; DL-α-lipoic acid

1. Introduction

Brain aging has become an area of intense research and a subject of much speculation fueled largely from the widely recognized fact that age is the biggest risk factor in most neurodegenerative diseases. According to the free radical theory of aging [1], the primary cause, which initiates the processes leading to the aging of an organism and its ensuing death, is uncontrolled production of free radicals. Free radicals once formed are countered by up-regulation of powerful antioxidant systems of the cell [2]. "Oxidative stress" results due to the loss of balance between ROS production and antioxidant defenses affecting all the vital organs resulting in aging. Free radicals have previously been reported to be capable of damaging many cellular components such as proteins, lipids and DNA [3–5].

The brain is particularly vulnerable to oxidative damage due to various factors like high utilization of inspired

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oxygen, high susceptibility of large amount of oxidizable polyunsaturated fatty acids that are prone to lipid peroxidation and the relative dearth of antioxidant defense systems [6,7]. Certain brain regions like cortex, striatum and hippocampus are highly enriched in non-heme iron, which is catalytically involved in the production of oxygen free radicals [8,9] thus increasing the risk of neurodegenerative disorders [10]. The main source of ROS is the mitochondria, where superoxide is produced during the process of electron transport [11]. The most important cellular targets for free radicals include phospholipids of cellular membranes, proteins and DNA, which eventually lead to death of the cell [12,13].

Lipid peroxidation is thought to proceed by radical mediated abstraction of the hydrogen atom from a methylene carbon on a polyunsaturated fatty acid side chain. Indeed damage to DNA and proteins may often be more important than damage to lipids in oxidative stress situations in vivo. Oxidative modifications of proteins in vivo may affect a variety of cellular functions involving proteins: receptors, signal transduction mechanism, transport systems and enzymes. The oxidative damage to proteins is reflected by increase in levels of protein carbonyls (PCO) [14]. Reaction of free radicals with the side chains of lysine, arginine, proline and glutamic acid residues of proteins leads to the formation of carbonyl derivatives [15]. These age-dependent changes appear to be unique to central nervous system since brain neurons are highly energetic cells with a high rate of production of reactive oxygen species damaging nuclear DNA.

The hydroxyl radical (·OH) typically formed by oxidation of a reduced heavy metal ion (Fe^{+} or Cu^{+}, usually) by hydrogen peroxide known as the Fenton reaction, may be the most dangerous because it can occur in the cell nucleus and lead to DNA damage. The damage to DNA is more variable, and attack by free radicals can produce DNA protein cross-links, modification of bases, etc. The DNA protein complexes generated by free radicals may be important DNA lesions exerting genotoxicity because these complexes are in general persistent and not readily repaired as that of other lesions [16,17].

Antioxidants that accumulate in brain and neuronal tissue are potential candidates for prevention or treatment of disorders involving oxidative damage. L-carnitine (β–OH–γ-trimethyl amino–butyrate) is a natural constituent of higher organisms. Carnitine is a quaternary ammonium compound that is water-soluble and biologically active in its L-isoform. Major function of carnitine includes the transport of long chain fatty acids in mitochondria for utilization in energy generation process [18] and also modulates intramitochondrial acyl-CoA/CoA ratio. Carnitine exhibits antioxidant activity to some extent [19,20] and shows signs of anti-apoptotic property [21]. Studies from our laboratory show that carnitine exhibits neuroprotective effects in aging animals [22]. The increased mitochondrial function and general metabolic activity without a concomitant increase in the free radical production can be facilitated in the presence of a potent antioxidant like DL-α-lipoic acid thus making it worthwhile in alleviating age-associated oxidative damage.

Thiol antioxidants in particular, may be good candidates for use in brain disorders. Lipoic acid and its reduced form DHLA has been staunchly established as a potent antioxidant because of its high singlet oxygen quenching capacity [23–25]. Lipoate can cross the blood brain barrier and is taken up by all areas of CNS and peripheral nervous system [26]. It is well recognized that DHLA accumulates in the lipid portion of membrane [27] that simultaneously scavenges free electrons thereby rendering them active protection. The present study was thus aimed to study the effectiveness of L-carnitine and DL-α-lipoic acid in curtailing macromolecular damage and increasing the activities of antioxidant enzymes in discrete brain areas of young and aged rats.

2. Materials and methods

2.1. Chemicals

L-carnitine, DL-α-lipoic acid and bovine serum albumin were from Sigma Chemical Company (St. Louis, MO). All other chemicals used were of analytical grade and were from Glaxo Laboratories, CDH Division, Mumbai, India and Sarabhai M. Chemicals, Boroda, India.

2.2. Experimental animals

The laboratory animal protocol used for this study was approved by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA) at IBMS, University of Madras, Chennai, India. Male albino rats of Wistar strain weighing approximately 130–160 g (young) and 380–410 g (aged) were used. They were healthy animals maintained and bred for >20 y at King's Institute of Preventive Medicine, Chennai. The animals were housed in large spacious cages under a daily photoperiod of 12-h light/dark cycle and were given food and water ad libitum throughout the experimental period and maintained at a temperature of 28±1 °C.

2.3. Grouping of animals

The animals were divided into 6 groups each group consisted of 6 animals. Group Ia were control young rats, Group Ib were young rats given L-carnitine, Group Ic were young rats given DL-α-lipoic acid, Group Id were young rats given L-carnitine and DL-α-lipoic acid, Group IIa were control aged rats, Group IIb were aged rats given L-carnitine, Group IIc were aged rats given DL-α-lipoic acid, and Group IId were aged rats given L-carnitine and