Abstract

Oxidative stress has been implicated in brain ageing and in age-related neurodegenerative disorders. Since N-acetylcysteine (NAC) has recently been shown to prevent oxidative damage in ageing brain, we have examined the effects of this thiolic antioxidant on the age associated oxidative stress related parameters in rat brain regions. The lipid peroxide formation, reduced glutathione (GSH) content along with the activities of superoxide dismutase (SOD) and catalase were determined in the cerebral cortex and cerebellum brain regions of the young (4 months) and older (14 months) female rats. The lipid peroxidation was observed to be increased in the cerebral cortex regions accompanied by simultaneous decrease in the GSH content in both the regions of older rats. The SOD activity was reduced in both the regions while catalase was reduced only in cerebellum region of the older rats. Following NAC supplementation (160 mg/kg. b. wt./ day), lipid peroxidation was observed to be reduced which was accompanied by enhanced GSH levels, along with enhanced SOD and catalase in both the brain regions of older rats. Further, in the younger rats the NAC treatment resulted in the decrease of lipid peroxidation in both the regions that was accompanied by the increase catalase activity in cerebral cortex region along with increase in GSH content and SOD in cerebellum regions. Our result suggests that the normal brain ageing is associated with the decrease in antioxidative defense status and the supplementation of thiol antioxidants like NAC may prove helpful in managing the age related brain disorders characterized by compromised antioxidative defense systems.

Key words: N-Acetylcysteine. Reduced glutathione. Lipid peroxidation. Antioxidative defense. Brain ageing.
Introduction

Ageing can be defined as the nonfunctional alteration of structure or homeostatic capability in an individual organism as it lives. Oxygen free radicals and oxidative events have been implicated as playing a role in bringing about the changes in cellular function that occur during ageing. The normal redox status of a cell which regulates several cellular processes such as signal transduction, gene expression, cell proliferation and cell death (neurotic and apoptotic) is usually maintained by the two opposing factors, antioxidants and oxidants produced during the normal cellular metabolism. Glutathione and thioredoxin are two such intracellular antioxidants in addition to other antioxidants obtained from the diet like Vitamin E, ascorbic acid and essential metals that help in normalizing the ageing induced alterations.

For a long time, dietary antioxidants are considered to be important means for fighting ageing processes. Hence, it is of utmost importance to know what kind of antioxidants and free radical scavengers could be most effective for ageing delay. Previously, we reported the use of N-acetyl cysteine (NAC) in reducing the oxidative stress in rat brains produced during lead exposure. NAC, a thiol compound having the formula C,H,O,S, is an excellent source of sulfhydryl (SH) groups that stimulates the reduced glutathione (GSH) synthesis, promotes detoxification and act directly as free radical scavengers. NAC have beneficial effects in conditions characterized by decreased GSH or oxidative stress such as HIV infection, cancer, heart disease and cigarette smoking. The sulfhydryl (SH) group is responsible for a great deal of the metabolic activity of NAC, while the acetyl-substituted amino group makes the molecule more stable against oxidation. SH groups are essential for defense against reactive oxygen species. So it is not surprising that NAC is a powerful scavenger of hypochlorous acid, and is capable of reducing hydroxyl radicals and hydrogen peroxide. However, progress in elucidating the evaluation of NAC efficacies on brain regions in modulating the age associated oxidative stress so far is slow. The present work is an attempt in this direction. For this, the age associated alterations on the lipid peroxides and antioxidant enzyme status in brain regions (cerebral cortex and cerebellum) were determined in two intracellular antioxidants in addition to other antioxidants obtained from the diet like Vitamin E, ascorbic acid and essential metals that help in normalizing the ageing induced alterations.

Materials and methods

Animals and treatments

Healthy female rats of the Wistar strain of 4 months old and 14 months old were obtained from the central animal house of Panjab University. The animals were housed in polypropylene cages under hygienic conditions and were provided standard animal feed and water ad libitum throughout the treatment duration of three weeks. All procedures were done in accordance with ethical guidelines for care and use of laboratory animals and were approved by the Animals Ethics Committee of Panjab University. For the present study, a total of 30 animals were used which were divided into two groups: age group I (4 months old) and age group II (14 months old) with 15 animals each. Both the groups were administered NAC (160 mg/kg b. wt., dissolved in physiological saline) intraperitoneally once a day for three weeks. Respective control of both the age groups were also run in parallel and were injected the same volume of saline. The animals were monitored regularly for their physical activity and general health and a weekly weight changes were recorded.

Tissue preparations

At the end of various treatments, the animals of each group were anesthetized with ether and sacrificed by decapitation; the brains were removed, rinsed in ice-cold isotonic saline, and cerebral cortex and cerebellum regions were dissected. A 10% (w/v) tissue homogenate was prepared in ice-cold 10 mM PBS (Phosphate-buffered saline, 0.15 M NaCl), pH 7.4. The homogenate was centrifuged at 1,000 g for 10 min. at 4 °C and the supernatant was used for biochemical assays. For the superoxide dismutase assay, the supernatant was further centrifuged at 12,000 g for 20 min.

Biochemical estimations

The quantitative measurement of lipid peroxidation was performed according to the methods of Wills. The malondialdehyde formed was measured by the reaction with thiobarbituric acid at 532 nm. The results were expressed as mmol malondialdehyde/ mg protein using the molar extinction coefficient malondialdehyde-thiobarbituric chromophore (1.56X 10^5 M^-1 cm^-1). The reduced glutathione (GSH) content was estimated according to the method of Ellman. In this method, 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) is reduced by -SH groups to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. The nitro mercaptobenzoic acid anion released has an intense yellow color and can be used to measure -SH groups at 412 nm. The superoxide dismutase (SOD) assay was performed according to the method of Kono. The extent of inhibition of reduction of Nitro Blue Tetrazolium (NBT) to blue formazan, by the addition of the enzyme was measured at 560 nm. The activity of enzyme was expressed as units/ mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 50%. Catalase was assayed according to the method of Luck, the breakdown of H_2O_2 being measured at 240 nm. The results were expressed as mmol H_2O_2 decomposed/ min/ mg protein. Proteins were estimated in the samples by the method of Lowry et al.
Statistical Analysis

Tabulated values represent means ± SD. One-way analysis of variance (ANOVA) and the Student-Newman-Keuls multiple comparison tests were used to analyze data from experimental and control groups. Values with p less than 0.05 were considered significant.

Results

In cerebral cortex region, lipid peroxides showed highly significant increase (p < 0.001) with age while no change was observed in cerebellar region when their respective controls are compared. The percentage increase in the lipid peroxides in cerebral cortex region in control of older rats (age group-II) was to an extent of 40.6% when compared to control of young rats (age group-I). However, NAC supplementation resulted in highly significant decrease (p < 0.001) in the lipid peroxides in both the cerebral cortex region and cerebellar region in both the age group-I rats (43.8% and 76.7% respectively) and age group-II rats (86.7% and 81.6% respectively) but to greater extent in older rats (See table I).

A significant decrease in the GSH content was observed in the control of age group II animals in both the cerebral cortex (57.2%) and cerebellum region (80.1%) of rat brains. After NAC supplementation to young animals, the glutathione was increased in the cerebellum region only to an extent of 25.4%, whereas, NAC treatment to age group II animals resulted in enhanced glutathione levels in both the cerebral cortex (207.4%) and cerebellum regions (146.8%) (See table II).

As a result of ageing SOD activity showed highly significant decline (p < 0.001) in cerebral cortex as well as cerebellum region. The older age group-II rats showed the decline in SOD activity to an extent of 49.2% in cerebral cortex and 71.5% in cerebellum region when its control is compared to control of younger age group-I rats. In cerebral cortex, the supplementation of NAC produced no significant change in SOD activity in younger age group-I rats, while in older age group-II rats it resulted a highly significant increase (p < 0.001) in SOD noted to an extent of 72.6%. Further, in cerebellum region both the age group-I and age group-II showed enhanced SOD activity following NAC supplementations. The percentage increase

<table>
<thead>
<tr>
<th>Lipid peroxides (n moles of MDA/mg of protein)</th>
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<tbody>
<tr>
<td>Regions</td>
</tr>
<tr>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>Cerebellum</td>
</tr>
<tr>
<td>Cerebellum</td>
</tr>
</tbody>
</table>

Statistical Analysis: Values are Mean ± S.D. of 6 determinations.
***p < 0.001: treated w.r.t. control.
###p < 0.001: age group-II w.r.t age group-I.

<table>
<thead>
<tr>
<th>Reduced glutathione content (GSH) (n moles of GSH/mg of protein)</th>
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</thead>
<tbody>
<tr>
<td>Regions</td>
</tr>
<tr>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>Cerebellum</td>
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</tbody>
</table>

Statistical Analysis: Values are Mean ± S.D. of 6 determinations.
***p < 0.001 : treated w.r.t. control.
###p < 0.001: age group-II w.r.t age group-I.
in SOD activity due to NAC treatment was observed to more extent in older age group-II (154.5%) than that of younger age group-I (30.1%) (See Table III).

The cerebral cortex showed no alterations in catalase activities while cerebellum region showed significant decline (p < 0.001) in catalase activity with age when the control of old age group II animals were compared with the control of young animals. NAC treatment in cerebral cortex region produced significant increase in catalase activity in both the age group-I (p < 0.05) and age group-II (p < 0.001) rats. The increase in catalase activity following NAC supplementation was found to more extent in older rats of age group-II (479.6%) than in young age group-I rats (45.6%). In cerebellar region NAC treatment to younger age group-I rats produced no significant change in catalase activity in while it resulted in highly significant increase (p < 0.001) in catalase activity in older age group-II rats (See Table IV).

Discussion

In multicellular organisms, ageing processes are associated with progressive degeneration of biological functions and increased susceptibility to diseases. Finding new treatments, and eventually cures, for the diseased conditions arising during ageing is therefore a priority. Damage due to free radicals is a major contributor to ageing.

Lipid peroxidation has been identified as a basic deteriorative reaction in the cellular mechanism of the ageing. Lipid peroxidation is initiated by the free radicals which oxidize the polyunsaturated fatty acids leading to the formation of conjugate dienes ultimately resulting in the production of hydroperoxides, cyclic peroxides and malondialdehyde. Acting as the first line of defense against the production of such hydroperoxides is a naturally occurring antioxidant glutathione (GSH), which is a major source of free thiol in most living cells. GSH in addition also participates in diverse biological processes such as the detoxification of xenobiotics and modulation of enzyme activity by disulphide interchange. In the present experiments, enhanced MDA levels were observed in cerebral cortex region of older age group, along with the decline in GSH content in both the brain regions, thus implicating the role of lipid pero-

### Table III

**Superoxide dismutase activity in the cerebral cortex and cerebellum regions of the younger (age group I) and aged (age group II) rat brains**

<table>
<thead>
<tr>
<th>Regions</th>
<th>Age group I</th>
<th>Age group II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>15.084 ± 1.64</td>
<td>15.873 ± 0.918</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>28.737 ± 3.424</td>
<td>37.397 ± 0.946***</td>
</tr>
</tbody>
</table>

Statistical Analysis: Values are Mean ± S.D. of 6 determinations.

*P < 0.001: treated w.r.t. control.

### Table IV

**Catalase activity in the cerebral cortex and cerebellum regions of the younger (age group I) and aged (age group II) rat brains**

<table>
<thead>
<tr>
<th>Regions</th>
<th>Age group I</th>
<th>Age group II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0.212 ± 0.028</td>
<td>0.310 ± 0.08'</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.997 ± 0.169</td>
<td>1.054 ± 0.16</td>
</tr>
</tbody>
</table>

Statistical Analysis: Values are Mean ± S.D. of 6 determinations.

*p < 0.05, "p < 0.01, ""P < 0.001: treated w.r.t. control.

**P < 0.001: age group-II w.r.t age group-I.
oxidation associated with simultaneous decline of GSH in the process of normal ageing. These results are in agreement with those of Leutner et al\textsuperscript{12}, Smith et al\textsuperscript{11} and Rodriguez-Martinez et al\textsuperscript{2} who observed the reaction products of active oxygen such as lipo- fuscin and lipid peroxide increases with ageing. These effects are due to continuous presence of small concentration of oxygen free radicals in the tissues during ageing\textsuperscript{11,12} which eventually leads to the imbalance in cellular redox status. In the present study, these changes were further reflected in the GSH concentration which decreased during ageing. Alterations in the normal GSH status influence the normal redox status of the cell and hence may lead to lipid peroxidation\textsuperscript{13}. Study in the ageing animal by Ravindranath et al\textsuperscript{14} and Hazelton and Lang\textsuperscript{15} had also indicated a low GSH content as a general phenomenon of aging brain and other ageing tissues.

The NAC supplementations to both the age groups proved beneficial in terms of lowering the MDA levels in brain regions accompanied by the increase levels of GSH in both the regions of older age group and in cerebellum region in case of younger rats. The decline in MDA levels due to NAC treatment is observed to greater extent in older age group (decline of 86.7\% in cerebral cortex and 81.6\% in cerebellum region). The increase in GSH levels following NAC supplementation in brain regions is due to the increased availability of cysteine, which is essentially required for the GSH synthesis\textsuperscript{16}. Experimental results by De Flora et al\textsuperscript{17}, Hoffer et al\textsuperscript{18} and Issels et al\textsuperscript{19} have also pointed the increases intracellular concentrations of GSH following NAC supplementations. The results reported here clearly indicates that the increase GSH concentration by NAC has contributed towards the decrease lipid peroxidation in the brain regions by improving the glutathione redox status which helps in maintaining the normal metabolic activity of the cell during ageing process.

Active oxygen species have been proposed to have been involved in the ageing process of body. Perez-Campo et al\textsuperscript{20} suggested the continuous presence of small concentrations of oxygen radicals in the tissues throughout the life span of animals to be contributing to ageing. SOD and catalase are both antioxidant enzymes that function as blockers of free radical process\textsuperscript{21}. In the present study, older age group showed significant decline in SOD activity in both the brain regions (decline of 49.2\% in cerebral cortex and 71.5\% in cerebellum), whereas, catalase showed significant decline only in the cerebellum region of its older age group (decline of 50.8\%). Semschi et al\textsuperscript{22} studied the age dependent activities of various enzymes involved in the protection against active oxygen species in rat brain and reported the decrease in enzyme activity as a result of reduced enzyme synthesis processes with age. The gradual decrease in catalase with increasing age appears to be due to an age-dependent change in the expression of their genes\textsuperscript{23}. Following NAC supplementation both SOD and catalase, showed significant increase in their activities in older age group. NAC through its thiol group acts as a scavenger of free radicals involved in the process of cellular ageing and therefore helps in maintaining the normal cellular metabolic state. The observed increase in antioxidant enzymes activity may be due to the direct consequences of the improved redox homeostasis following NAC administration and, resulting into the better enzymes synthesis in older age group.

In summary, the results from the present study suggest that supplementation of extraneous antioxidants and free radical scavengers like NAC may be useful in combating the free radical induced harmful effects during ageing processes.

References


