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EXPERIMENTAL STUDY

Anti-oxidative effect of resveratrol on aluminum induced toxicity in rat cerebral tissue

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ABSTRACT
INTRODUCTION: The direct protective effects of resveratrol against oxidative stress have been demonstrated in neuroglial cells, the mechanisms of these effects are not fully understood. The aim of this research was to study the effect of resveratrol on AL induced cerebral injury in rat.
METHODS: We divided the groups as follows with 10 animals each: a) Group I – served as control receiving normal drinking water and diet ad libitum. b) Group II – animals were administered aluminum at a dose level of 100 mg/kg body weight for a period of 6 weeks daily through oral gavage. c) Group III – animals were administered aluminum at a dose level of 100 mg/kg body weight and resveratrol at a dose of 10 mg/kg body weight intraperitoneally for a period of 6 weeks daily. After 6 weeks rats were anesthetized and decapitated. Brains were removed immediately and frozen in liquid nitrogen
RESULTS: The levels of SOD and GPx antioxidant enzymes were decreased in all of the groups receiving aluminium, but it was less severe in resveratrol treated group. SOD and GPx levels in aluminium + resveratrol group were higher than in the aluminum group ($p < 0.05$). MDA level, as an index of lipid peroxidation, increased significantly in all of the groups receiving aluminium. MDA level was lower in aluminium + resveratrol group compared to aluminum group and the difference was significant ($p < 0.05$).
CONCLUSIONS: This study suggests that resveratrol is effective in preventing AL induced toxicity by reducing MDA production in cerebral tissue. Resveratrol also attenuated SOD and GPx suppression in cerebral tissue significantly. Our findings provide the rationale for further studies directed to understanding the mechanism of resveratrol in preventing neurodeterioration *(Tab. 1, Ref. 35)*. Text in PDF *www.elis.sk.*
KEY WORDS: aluminum, brain, resveratrol.

Introduction

There is an increasing evidence in the literature that during environmental stress reactive oxygen species (ROS) can cumulatively damage biological molecules, leading to structural and functional deterioration of cells during the aging process (1).

Aluminium (Al) is the third most abundant element on the earth crust and gets an easy access to our body through use of cooking utensils, deodorants, antacids, etc. Al is routinely used as a water treatment reagent and is often added in the processing of food and pharmaceutical products (antacids) (2). Although aluminum (Al) is a relatively low redox mineral, it can induce oxidative damage through multiple mechanisms. It can bind to negatively charged brain phospholipids, which contain polyunsaturated fatty acids and are easily attacked by reactive oxygen species (ROS) such as $O_2^{\cdot -}$, H_2O_2, $OH^{\cdot}$, and OH^- (3). Aluminum can also stimulate iron-initiated lipid peroxidation in the Fenton reaction, which causes ROS production and Fe^{3+} formation. Superoxide ($O_2^{\cdot -}$) is neutralized by Al^{3+} to form an Al-$O_2^{\cdot -}$ complex, which increases the oxidative capacity of $O_2^{\cdot -}$ (4). Aluminium has been reported to alter blood-brain barrier (5) and gets deposited in the cortex, cingulat bundles, corpus callosum (6) and hippocampus. (7) Aluminium being an inert metal has been suggested to induce oxidative damage indirectly by potentiating the peroxidative effect of Fe^{2+} (8). AL generates reactive oxygen species, resulting in oxidative deterioration of lipids, proteins and DNA. Therefore, the estimation of free radical generation and antioxidant defense has become an important aspect of investigation in mammals (9).

Resveratrol (trans-3,49,-5-trihydroxystilebene), a natural phytoalexin compound found in various plants such as grapes and berries, is known to have potent anti-oxidant and anti-tumorigenic activities (10). Many studies have shown that resveratrol can prevent or slow the progression of a variety of conditions, including cancers, cardiovascular diseases, or ischemic injuries and can enhance stress resistance and extend lifespan (11). Resveratrol also promotes antioxidant defense by regulating a host of antioxidant enzymes (12). Moreover, it has been demonstrated that resveratrol has beneficial effects in neurological diseases (13) and is able to inhibit amyloid peptide neurotoxicity (14). Whilst direct protective effects of resveratrol against oxidative stress have been demon-

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strated in neuroglial cells, the mechanisms of these effects are not fully understood (14). The aim of this research was to study the effect of resveratrol on AL induced cerebral injury in rat.

Materials and methods

Healthy male Wistar rats were purchased from central animal house of pastor. The animals were acclimatized in the departmental animal house for 2 weeks in plastic cages and were provided feed and water ad libitum. The rats were monitored for their health and body weight. The animals were kept and cared for and during all stages in compliance with the applicable guidelines and regulations of the institute. We divided the groups as follows with 10 animals each:

(a) Group I – served as control receiving normal drinking water and diet ad libitum.

(b) Group II – animals were administered aluminum at a dose of 100 mg/kg body weight for a period of 6 weeks daily through oral gavage (15).

(c) Group III – animals were administered aluminum at a dose of 100 mg/kg body weight and resveratrol at a dose of 10 mg/kg body weight intraperitoneally for a period of 6 weeks daily (16). After 6 weeks rats were anesthetized and decapitated. Brains were removed immediately and frozen in liquid nitrogen.

Assay of antioxidant enzymes

The cerebral tissue was frozen in liquid nitrogen and stored at –80 °C until further preparation. In order to measure anti-oxidant enzyme activity, the cerebral samples were homogenized in 1.15 % KCl solution. Superoxide dismutase (SOD) activity in liver tissue was determined by using xanthine and xanthine oxidase to generate superoxide radicals which then react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction (Ransod, Randox Laboratories Ltd., Antrim, United Kingdom). Results were obtained as SOD Unit/mg protein (17).

Glutathione peroxidase (GPx) activity in cerebral tissue was measured using the method described by Paglia and Valentine. GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured (Ransod, Randox Laboratories Ltd., Antrim, UK). Results were obtained as GPx Unit/mg protein (18).

Tissue MDA level

Tissue malondialdehyde was determined by the method of Uchiyama and Mihara (19). 3-mL aliquot of 1 % phosphoric acid and 1 mL of 0.6 % thiobarbituric acid solution were added to 0.5 mL of 10 % tissue homogenate. The mixture was heated in boiling water for 45 minutes. After cooling, the color was extracted into 4 mL of n-butanol. The absorbance was measured in a spectrophotometer (Amersham Pharmacia Biotech UK Ltd., Little Chalfont, Buckinghamshire, UK) at 532 nm ($\acute{\varepsilon} = .56\times10^5$ mol/$L^{-1}cm^{-1}$). The amounts of lipid peroxides calculated as thiobarbituric acid reactive substances (TBARS) of lipid peroxidation were expressed as nMol/ml (20).

Tab. 1. The effect of resveratrol on antioxidant enzymes content and MDA levels in rat cerebral tissue.

	Control	Aluminum	Aluminum+ Resveratrol
Cerebral GPx	5.07±.49	3.18±0.51	3.7700±0.26
Cerebral SOD	4.41±.78	3.02±0.22	3.7700±0.49
Cerebral MDA	4.03±.65	6.83±0.71	4.9300±0.52

The values are shown as mean ± SD for rats in each group and difference of ($p < 0.05$) considered significant. SOD – superoxide dismutase, GPx – glutathione peroxidase, MDA – malondialdehyde

Statistical analysis

Data were expressed as means ± SD. Differences among various groups were tested for statistical significance using the one-way ANOVA test and Tukeys post test. A p value of less than 0.05 denoted the presence of a statistically significant difference.

Results

SOD and GPx level

The levels of SOD and GPx antioxidant enzymes were decreased in all of the groups receiving aluminium, but it was less severe in resveratrol treated group. SOD and GPx levels in aluminium + resveratrol group were higher than in the aluminum group ($p < 0.05$) (Tab. 1).

MDA level

MDA level, as an index of lipid peroxidation, increased significantly in all of the groups receiving aluminium. MDA level was lower in aluminium + resveratrol group compared to aluminum group and significantly ($p < 0.05$).

Discussion

Oxidative stress is associated with an increase in oxidizing species that destruct the vascular and neuronal cells in central nervous system. Oxidative stress is due to the imbalance between the oxygen free radicals generated and the antioxidant defense system to detoxify the reactive intermediates (21). Oxidative stress changes the signaling pathways that may induce cellular responses such as inflammation, cell proliferation, and cell survival and death (22). Reactive oxygen species (ROS) are chemically reactive molecules that consist of oxygen ions and peroxides that include hydrogen peroxide, singlet oxygen, nitric oxide, peroxynitrite, and superoxide free radicals. The release of peroxides and free radicals is toxic to the cell, which may lead to cell death. The antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and peroxidases, and non-enzymatic free radical scavengers (ascorbic acid, α-tocopherol, and GSH) convert the reactive oxygen species to water and oxygen, the stable molecules. These antioxidants are known to protect the cells and tissues against oxidative injury caused by reactive oxygen species (23).

Superoxide dismutase (SOD), an oxygen radical scavenger, which converts the superoxide anion radical present in the upper stream of reactive oxygen metabolism cascade, will afford protection from cell damage (24). SOD catalyzes the dismutation of the superoxide anion (O_2^-) into H_2O_2; GSH-Px is a selenoprotein, which reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing GSH (25). In our study, treatment with AlCl3 caused a significant increase in brain MDA and decrease in cerebral GPx and SOD activities (Tab. 1). Similarly El-Demerdash (26) reported that the lipid peroxidation significantly increased during Al exposure and this was used as a marker of Al toxicity. On the other hand, Nayak et al (27) found that AlCl3 caused a significant decrease in the levels of GPx and SOD, indicating that Al decreases antioxidant defense system. In our study resveratrol administration decreased MDA elevation in rats receiving both AL and resveratrol. Resveratrol also attenuated SOD and GPx suppression by AL administration significantly.

Both Jutka and Gill (28), and Gupta and Shukla (29) observed that al administration lowered SOD and GPX activities in the brains of rats. It is noteworthy that increased lipoperoxidation was also found by these authors in brain tissues. Similar results have been reported by Chainy et al (30) in the liver, and by Verstraeten et al (31) in the brain myelin of mice. Taken together, the preceding results suggest that A(promotes oxidative stress by decreasing the activity of free radicals scavenging enzymes such as SOD and GPX, a biological effect confirmed by increased accumulation of lipoperoxidation products.

Moumen et al (32) reported that after Al administration GPX is decreased in brain tissue, but they found increased SOD activity in brain tissue when most authors report opposite results in a similar setting. They had already found a similar change in the plasma of SALS patients (33) (increased SOD and decreased GPX activities), while RBC SOD activity is unchanged in these patients (34). It was then suggested that, at least in plasma, this increase reflected predominantly Ec SOD (35). Such an interpretation is difficult to apply to the brain, unless the extraction method chosen here would artificially enhance Ec SOD extraction. A more appealing explanation could be that AL administration induces a biphasic response in SOD, a more appealing explanation could be that AL administration induces a biphasic response in SOD with an initial enhancement of activity followed by a decrease. The duration of administration was shorter in Moumen et al (32) study (1 week) than in that of Jutka and Gill (28) (2 weeks) and that of Gupta and Shukla (10) (29 months).

Conclusions

This study suggests that resveratrol is effective in preventing AL induced toxicity by reducing MDA production in cerebral tissue. Resveratrol also attenuated SOD and GPx suppression in cerebral tissue significantly. Our findings provide the rationale for further studies directed in understanding of mechanism of resveratrol in preventing neurodeterioration.

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