THE EFFECT OF L-TYROSINE, VITAMINS E AND C ON PERPHENAZINE-INDUCED CATATONIA IN RAT

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Abstract

Catatonia is one of the major signs of parkinson’s disease which is due to impaired dopamine in extrapyramidal system. This study was performed to elucidate the role of L-tyrosine, vitamin E and vitamin C on perphenazine-induced catatonia in model of rat. Eight groups of rats were pretreated orally by a single dose of L-tyrosine (1500 mg/kg), vitamin E (250 mg/kg), vitamin C (300 mg/kg), vitamin E plus vitamin C, vitamin E plus L-tyrosine, vitamin C plus L-tyrosine, vitamins E and C plus L-tyrosine and normal saline (control group) for two weeks, respectively. One hour after the last dose, perphenazine (5 mg/kg) was administered (IP) to induce catatonia. Muscular rigidity of animals was determined by scoring method of Morpurgo. The results of this study indicate that L-tyrosine plus vitamins E and C could reduce the perphenazine-induced muscular rigidity better than other groups.

Keywords:
L-tyrosine, Vitamin E, Vitamin C, Perphenazine, Catatonia, Rat.

Introduction

A variety of medication options and medical strategies have been proposed as a means of Parkinson’s disease (PD) treatment. On the other hand, the problems of disease progression and levodopa therapy complication have become a major focus of investigations. However, further study of existing drugs and the introduction of new drugs, have improved the treatment of PD. According to free-radical hypothesis and endogenous toxins in etiology of PD, it seems that reducing the generation and collection of free-radicals can decrease the disease progress (1). A number of studies suggest the involvement of oxidative stress in the substantia nigra from parkinsonian patients. If oxidative stress is relevant in the pathogenesis of PD, the consumption of antioxidant substances in the diet could theoretically influence the risk for this disease (2,3). The prominent pathological feature of brain in PD is the selective degeneration of dopaminergic neurons in substantia nigra of the midbrain (4). In PD, the dopamine neuronal cell death in the nigrostriatal system has been proposed to be mediated by reactive oxygen radicals such as hydroxyl radicals (5). Severe oxidative stress progressively leads to cell dysfunction and ultimately cell death. Oxidative stress is defined as an imbalance between pro-oxidants or free radicals, and anti-oxidizing systems. The oxygen required for living may indirectly be responsible for negative effects. These deleterious effects are due to the production of free- radicals, which are toxic for the cells (superoxid anions, hydroxyl radicals, peroxy radicals, hydrogen peroxide, hydroperoxides and peroxinitrite anions). Free radical attacks are responsible for cell
damage and the targeted cells are represented by the cell membranes, which are rich in unsaturated fatty acids, sensitive to oxidation reactions. DNA is also the target of severe attacks by the reactive oxygen species (6,7). The free-radicals scavenging or neutralization system of these reactive oxygen species use a collection of mechanisms e.g. vitamins E and C, enzymes (superoxide dismutase, glutation peroxidase and glutathione reductase), capable of neutralizing reactive oxygen species. The efficacy of this system depends on the genome for the enzymatic defense systems and intake of vitamins via foods (6). In this study the effects of L-tyrosine and vitamins E and C as antioxidant were invstigated.

Materials and methods
Sprague-Dawley rats of either sex, weighing 180 to 200g were used during the study. Animals were kept in a clean holding room on a 12 h light and dark cycle, with relative humidity of 45% to 55%. Temperature was set at 23±2°C. Animals had access to unlimited standard concentrated food pellets (Pars Khurakdam-Shushtar, Iran) and tap water ad libitum.

The animals were randomly divided into eight groups (n=8) and were pre-treated orally with a single dose/day of L-tyrosine (1500 mg/kg), vitamin E (250 mg/kg), vitamin C (300 mg/kg), vitamin E plus vitamin C, vitamin E plus L-tyrosine, vitamin C plus L-tyrosine, vitamins E and C plus L-tyrosine and normal saline as the vehicle of all drugs (control group) for two weeks, respectively. One hour after last oral administration, perphenazine (5 mg/kg) was administered (IP) and relative muscular rigidity was determined at 20, 40, 60, 90, 120, 180 and 240 min after injections. Muscular rigidity was determined using the method of Morpurgo (8). The development of catatonia was observed and scored as follows: Stage 1, rat moves freely when placed on the table, score allocated = 0; Stage 2, rat moves only when touched or pushed, score allocated = 0.5; Stage 3, rat placed on the table with one of the front paws alternately on a 3cm high block. Fail to correct the posture in 10 seconds, score allocated = 0.5 for each paw with a total score of 1 for this stage. Stage 4, rat fails to correct the posture in 10 seconds when the front paws are placed alternately on a 9cm high block, score allocated = 1 for each paw with total score of 2 for this stage. Thus, for a single rat, the maximum possible score would be 3.5 reflecting total catatonia. Low score would mean an apparently lower degree of catatonia.

Statistical analysis
Results of treatment effects were statistically analyzed using kruskal-wallis nonparametric test and willcoxon matched-pairs test. Significant differences were considered when P<0.05.

Results
A) L-tyrosine treated group in comparison with control group (normal saline) showed significant difference (p<0.05) in all specified times of trial except 20 and 40 min (Fig. 1).
B) Vitamin E treated group in comparison with control group had no significant difference in any times of trial except the 60 min (Fig. 2).
C) Vitamin C treated group in comparison with control group had no significant difference in all specified times of trial (Fig. 3).
D) L-tyrosine plus vitamin E treated group in comparison with L-tyrosine treated group had no significant difference in all specified times of trial (Fig. 4).
E) L-tyrosine plus vitamin C treated group in comparison with L-tyrosine treated group had no significant difference in all specified times of trial (Fig. 5).
F) L-tyrosine plus vitamins E and C treated group in comparison with L-tyrosine treated group showed significant difference (p<0.05) in 20 and 40 min of trial (Fig. 6).
Discussion
Parkinson’s disease is an age-related disorder characterized by progressive degeneration of dopaminergic neurons in substantia nigra and corresponding motor deficits (9).

Fig. 1: Comparison of perphenazine-induced muscular rigidity between L-tyrosine treated and control group. * indicates significant difference (P<0.05).

Fig. 2: Comparison of perphenazine-induced muscular rigidity between vitamin E treated and control group. * indicates significant difference (P<0.05).
The etiology of PD remains unknown, making it difficult to develop therapeutic approaches to stop the progression of the disease. The best known treatment to date is based on the use of L-dopa or dopaminergic agonists. These are merely substitutive therapies and have limitations because of their side effects. Thus, the development of new therapeutic strategies will require a far better knowledge of the mechanism and the consequences of nerve cell death in PD (10).

Fig. 3: Comparison of perphenazine-induced muscular rigidity between vitamin C treated and control group.

Fig. 4: Comparison of perphenazine-induced muscular rigidity between L-tyrosine plus vitamin E treated and L-tyrosine group.
Aging is a major risk factor for neurodegenerative disease including PD. An unbalanced overproduction of reactive species may give rise to oxidative stress which can induce damage, ultimately leading to neuronal death by apoptosis or many evidences indicate that oxidative stress is involved in pathogenesis of PD (11). Vitamin E is the most important lipid soluble antioxidant. Since its discovery, studies of the constituent tocopherols and tocotrienols have focused mainly on their antioxidant properties (12).

Fig. 5: Comparison of perphenazine-induced muscular rigidity between L-tyrosine plus vitamin C treated and L-tyrosine group.

Fig. 6: Comparison of perphenazine-induced muscular rigidity between L-tyrosine plus vitamins (E and C) treated and L-tyrosine group. * indicates significant difference (P<0.05).
In 1991 it was first described non-antioxidant cell signaling functions for alpha-tocopherol, demonstrating that vitamin E regulates protein kinase C activity in smooth muscle cells (12). At the transcriptional level, alpha-tocopherol modulates the expression of liver collagen alpha gene, collagenase gene and alphatropomyosin gene. Recently, a tocopherol-dependent transcription factor (tocopherol-associated protein) has been discovered. In cultured cells it has been demonstrated that vitamin E inhibits inflammation, cell adhesion, platelet aggregation and smooth muscle cell proliferation. Recent advances in molecular biology and genomic techniques have led to discovery of novel vitamin E-sensitive genes and signal transduction pathways (12,13).

Vitamin E as an antioxidant has been employed in treatment of neurodegenerative disorders associate with oxidative stress (14). It has been shown that the risk of PD is reduced among men and women with high intake of vitamins E and C (15). Vitamin C is essential for the normal functions of living cells and involved in many enzymic reactions. Vitamin C is a coenzyme required for development of collagen, teeth and bones, for wound healing, aiding the absorption of iron from intestine, synthesis of dopamine, noradrenaline and adrenaline. Carnitine also needs vitamin C for transfer of energy to the mitochondria. Vitamin C is an antioxidant, acting to lessen oxidative stress and acts as a substrate for ascorbate peroxidase (16).

The results of this study exhibited that muscular rigidity decreased in group which received L-tyrosine plus vitamins E and C in comparison with group received L-tyrosine alone. The group that received only vitamin C in comparison with control (saline) group did not show any significant decrease in muscular rigidity in all specified times of trial. Vitamin C is a weak antioxidant, but when administered with vitamin E, the combination showed a stronger antioxidant effect in comparison with group that received only vitamin E. Such effect has been shown to increase the cognitive function of mouse. Perhaps it may be due to ability of vitamin C to regenerate α-tocopherol from tocopherol radical (17, 18).

Epidemiological data suggest that antioxidants may have a beneficial effect on many age-related disease including neurodegenerative diseases (6). Interest in the relationship between antioxidants and PD led to a preliminary trial using high amounts of vitamin C and vitamin E in early PD and to a long ten-year controlled trial of high amounts of vitamin E combined with the drug selegiline. In the trial combining vitamins C and E, people with early Parkinson’s disease given 750 mg of vitamin C and 800 IU of vitamin E four times a day (totaling 3000 mg of vitamin C and 3200 IU of vitamin E per day) were able to delay the need for drug therapy (i.e., L-dopa or selegiline) by an average of about two and a half years, compared with those not taking the vitamins. The ten-year controlled trial used 2000 IU of vitamin E per day found no benefit in slowing or improving the disease. The difference in the outcomes between these two trials might be due to the inclusion of vitamin C and/or the higher amount of vitamin E used in the successful trial (19,20).

Although current data indicate that the antioxidants can not prolong maximal life span, the beneficial impact of antioxidants on various aged-related degenerative diseases may forecast an improvement in life span and enhance quality of life. The results of this study confirmed the clinical outcomes of the use of combined antioxidants mentioned earlier. The combined anti-oxidants and L-tyrosine may also be effective to delay or prevent the PD in late ages. However, this hypothesis needs to be tested clinically.
References