L-DOPA, also known as Levodopa and L-3,4-dihydroxyphenylalanine (1), is best known to the world as a treatment for the neurological disorder – Parkinson’s disease (PD). L-DOPA is commonly derived from its natural plant source (2-4). L-DOPA is the precursor for the neurotransmitter dopamine (5), and is produced by the action of the enzyme tyrosine hydroxylase on the amino acid tyrosine (5). Earlier results have suggested that L-DOPA and dopamine might have a complex mixture of pro- and anti-oxidant effects contributing to tissue damage due to oxidative stress. Furthermore, entacapone, a COMT inhibitor, is known to retain greater levodopa levels in plasma during coadministration. Hence, the role of L-DOPA + entacapone in aluminum induced oxidative stress in the rat brain was evaluated. Sprague Dawley rats (n = 6/group) were treated with either the vehicle, aluminum, L-DOPA + entacapone or aluminum + L-DOPA + entacapone for 28 days. The intact brains were processed for lipid peroxidation (LPO) and superoxide dismutase (SOD) activity. Aluminum treatment showed highly elevated levels of LPO while the combination of L-DOPA and entacapone could not control this oxidative burst in the rat brains both in presence and absence of aluminum. No change was observed in the brain or the circulating SOD activity. Hence, it is derived that protective role of L-DOPA in AD management is not exerted through its antioxidant property and may be manifested due to its involvement in other pathways.

Keywords: Alzheimer’s disease, aluminum, entacapone, L-DOPA
Deficits and stereotypies in AD may be due to changes in the dopaminergic system as they could be ameliorated by levodopa treatment and hence this may have a therapeutic significance in AD (12).

In AD, there are a number of contributory sources that are thought to play an important role in the imposition of oxidative stress like increased iron in a redox active state in the neurons, increased NO synthesis in the microglia, abnormalities in the mitochondrial genome and so on. Also lipid peroxidation, a hallmark of oxidative tissue injury, has been found to be elevated in the AD brain (13). Hence, it is speculated that the agents that prevent oxidative damage will be particularly efficacious in the treatment for Alzheimer’s disease.

A number of environmental factors have been put forward as possible contributory causes of Alzheimer’s disease and aluminum is identified as one of these factors (15). The possible mechanisms of aluminum induced neurotoxicity have been related to cell damage via free radical production (16). Aluminum has been shown to be associated with both plaques and tangles in AD (13). Various investigations have suggested that Alzheimer’s disease is more common in areas where the aluminum content in water supplies is the highest (15). Hence, aluminum induced oxidative stress in the rat brain is a fairly good and commonly utilized animal model to mimic human AD like symptoms (17-19). Hence, the current study was aimed at studying the effect of L-DOPA in combination with entacapone on aluminum induced oxidative stress in the in vivo rat model.

**EXPERIMENTAL**

**Chemicals**

Aluminum chloride (AlCl₃) was obtained from SD Fine Chemicals Ltd., Mumbai, India. L-DOPA was purchased from Hi Media, Mumbai, India and entacapone was a gift from R. A. Chemical and Intermediates Pvt. Ltd., Hyderabad, India.

**Animals**

Sprague Dawley rats (n = 24) of either sex, with an average weight of 198 ± 8 g, bred in the animal colony at the B. V. Patel PERD, Centre, Ahmedabad, from the original stock obtained from National Institute of Nutrition, Hyderabad, were used for the study. The animals were housed in temperature controlled rooms (27 ± 1°C), at 10% air exchange with a relative humidity of 60 ± 5% at light:dark regimen of 14:10 h. Amrut certified rodent diet (Maharashtra Chakan Oil Mills Ltd.) and tap water (boiled water cooled to room temperature) was provided ad libitum to the experimental animals.

The experimental protocol was approved by the Institutional Animal House Ethics Committee, constituted by the Ministry of Social Justice and Empowerment, Government of India, prior to the initiation of the experiment.

**Dosage regimen**

Animals were divided into the following treatment groups: 1. Vehicle Control (normal saline) 2. Disease Control: Aluminum chloride - 4.2 mg/kg body weight, i.p. 3. Test 1: L-DOPA (4.17 mg/kg) + Entacapone (6.67 mg/kg body weight p.o.) 4. Test 2: Aluminum chloride - 4.2 mg/kg body weight, i.p. + [L-DOPA (4.17 mg/kg) + Entacapone (6.67 mg/kg body weight p.o.)]. Dose of aluminum was taken as per earlier experiments (15) and dose of L-DOPA and entacapone were calculated taking human dose of 250 mg/day and 400 mg/day, respectively.

**In vivo treatment**

Animals in all the groups were dosed for 28 days according to the above treatment (n = 6/group). Animals were euthanized on the 29th day and the intact brain was removed. It was washed with saline to clean traces of blood and divided into two halves. They two processed further for LPO estimation and SOD activity, respectively.

**Estimation of lipid peroxidation**

The first half of the brain was weighed and taken in 5 mL of Hank’s balanced salt solution (HBSS, pH 7.4) and homogenized at 3500 rpm using a Polytron homogenizer (Kinematica, Switzerland) (3 cycles of 30 s each). The homogenate was then centrifuged at 3500 rpm (500 × g) for 10 min. The pellet was resuspended in 0.1 mL of HBSS that was then used for estimation of lipid peroxidation. Lipid peroxidation was measured in terms of malonaldehyde (MDA):thiobarbituric acid (TBA) reaction as reported by Okhawa et al. (15). Appropriate controls were used at different steps during this estimation.

**Assay of superoxide dismutase activity**

The other half of the brain was taken in 4 mL of chilled Tris buffer 50 mM (pH 8.2, with 2 mM EDTA) and homogenized at 13,000 rpm (3 cycles of 30 s each) using a Polytron homogenizer. The homogenate was then centrifuged at 15,000 rpm at 4°C for 30 min
Using Sorval high-speed centrifuge (Sorval, USA) with fixed angle rotor (SS34). The supernatant was used for the assay of superoxide dismutase (SOD) activity by the method of Marklund and Marklund (16). All calculations were made as per gram fresh weight.

**Circulating Superoxide Dismutase**

On the 29th day blood was collected by cardiac puncture in heparinized disposable syringes. It was then centrifuged at 4000 rpm for 7 min at 4°C and plasma was separated and stored at -70°C until further assay for superoxide dismutase activity as detailed above. The remaining RBCs were then subjected for extraction of superoxide dismutase.

**Extraction and Activity of Superoxide Dismutase from Red Blood Cells**

Heparinized blood was centrifuged at 2500 rpm for 30 min at 4°C and the plasma was carefully separated. Normal saline (0.9%, 5 mL) was added to the erythrocyte pellet and this was transferred to 15 mL centrifuge tubes. After the cells were washed thrice with saline as above, they were diluted with 4 mL of double distilled water to lyse the erythrocytes. Ethyl alcohol (1 mL) and chloroform (0.6 mL) were added to separate the hemoglobin. The tubes were shaken vigorously for 15 min and centrifuged at 2500 rpm for 10 min at 4°C. Water ethanol layer was aspirated and diluted with 0.7 mL of double distilled water. Superoxide dismutase activity was measured in the aqueous-alcohol layer as described above (22).

**Statistical analysis**

All results have been presented as the mean ± SEM. Statistical analysis has been performed using one way ANOVA followed by Dunnet’s test and p < 0.05 has been considered significant.

**RESULTS**

*In vivo* changes in lipid peroxidation (LPO) (expressed as MDA levels) and SOD activities in different groups of rat brain homogenate have been shown in Figure 1. MDA levels were found to be highly elevated in the aluminum treated group as compared to the vehicle control (p < 0.05). Further, L-DOPA and entacapone in combination also gave a pro-inflammatory response and lead to a significant increase in the MDA levels (p < 0.05 vs. vehicle control). Moreover, the aluminum plus L-DOPA and entacapone treated group also showed an elevated MDA value when compared to the vehicle control (p < 0.05). However, no significant changes were observed in the SOD activity in any of the groups. Similarly circulating superoxide dismutase levels also did not show any significant change amongst the groups (data not presented).

**DISCUSSION AND CONCLUSION**

Levodopa, the metabolic precursor of dopamine, crosses the blood brain barrier and presumably is converted to dopamine in the brain. This is thought to be the mechanism whereby levodopa relieves symptoms of Parkinson’s disease including tremors, stiffness, and slowness of movement (7). It is currently understood that when entacapone is given in conjunction with levodopa, the plasma levels of levodopa are greater and more sustained than after administration of levodopa alone. It is believed that at a given frequency of levodopa administration, these more sustained plasma levels of levodopa result in more constant dopaminergic stimulation in the brain, leading to greater effects on the signs and symptoms (7). Hence, in the current study levodopa has been used in a combination with entacapone to enhance the effect of levodopa.

Previous experiments in Alzheimer’s patients have shown no change in serum free radical load as well as stable antioxidant values in the serum as compared to the control patients (23). When levodopa was used as a treatment for senile dementia in patients for two weeks in gradually increasing dosage regimen, a significant improvement in the intellectual quotient was observed in the patients, however, without any change in the behavioral indices (24). Also reports of small but consistent improvements in specific memory functioning in Parkinson’s disease with dopamine replacement therapy support the contention that dopaminomimetics may have some circumscribed use for attenuating the symptoms of dementing illness, although probably not as monotherapy (25). Hence, enough clinical evidences exist towards the implications of L-DOPA in Alzheimer’s disease.

In our experiment L-DOPA in combination with entacapone could not in itself lower the oxyradical load existing in a normal rat brain and instead caused an increase in the LPO levels. Further, when challenged with a free radical generating system in the aluminum treated animals, L-DOPA failed to demonstrate any antioxidant properties. Earlier results have suggested that L-DOPA and dopamine might have a complex mixture of pro- and anti-oxidant effects (6). Hence, in this case we can derive that L-DOPA is indeed acting in a pro-inflammato-
ry manner under both normal and oxyradical stressed conditions in the rat brain.

Thus, the results obtained here show that L-DOPA and entacapone combination does not exert its neuroprotective effect (if any) through the oxyradical antioxidant mechanism and may be demonstrating its activity during AD via certain other pathways. Hence, further studies pertaining to the detailed mechanism of action of L-DOPA in AD need to be carried out.

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