Anti-Depressant and Neuroprotective Effects of Captopril and Perindopril in Paraquat Model of Parkinsonism

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Non-motor symptoms such as depression, dementia, autonomic nervous system problems may be more evident in the later part of Parkinsonism. L-dopa is largely ineffective for non-motor symptoms. The objective of the present study was to evaluate the anti-depressant and neuroprotective role of captopril and perindopril in paraquat mice model of Parkinsonism. Adult Swiss albino mice were divided into five groups of six each. Parkinsonism was induced with paraquat (7mg/kg bodyweight at an interval of 2 days) in four groups. Experimental group was treated with captopril (20mg/kg intraperitoneal) and perindopril (5mg/kg intraperitoneal). Depression influences on behaviour was studied with forced swim test and tail suspension test. Oxidative stress markers – glutathione, lipid peroxidation assay, myeloperoxidase activity, catalase, superoxide dismutase, monoamine oxidase A and Bare carried out in one hemisection of the mice brain to evaluate the neuroprotective role of the test drugs. The test group mice exposed to captopril and perindopril had significantly less immobility time in both forced swim test and tail suspension test in comparison to the paraquat group, indicating anti-depressant effects of these drugs. Lipid peroxidation, myeloperoxidase activity, catalase, superoxide dismutase, monoamine oxidase B levels were significantly increased in both captopril and perindopril groups in comparison to the control group. Captopril and perindopril have shown beneficial effects for depression (as evidenced through forced swimming test and tail suspension test) in paraquat model of Parkinsonism. These drugs reduce the oxidative stress in paraquat mice model of Parkinsonism.

Keywords: tail suspension test; forced swim test; depression; Parkinsonism; oxidative stress.

Routine management of Parkinsonism essentially concentrates on the commonly occurring motor symptoms. As the disease progresses, dyskinesia and falls, midline symptoms complicate the management of PD. Non-motor symptoms such as depression, dementia, autonomic nervous system problems may be more evident in the later part of the disease[1-3]. To addresses these later problems, researchers around the world are looking for newer drug targets that reserves or at least halts the disease process. The therapeutic targets that are assumed to involve in striatonigral neuronal injury and dysfunction are centre of attraction. Molecules that alter á-synuclein aggregation,[4] drugs that influences the striatal nitric oxide- guanylylcyclasesreceptors,[5]nuclear

factor erythroid-2-related factor 2,[6] mixed lineage kinase (MLK) –c jun N terminal kinase (JNK) signaling cascade,[7]glucanon like peptide -1 (GLP-1) agonists, p75 neurotrophin receptor-sortilin signalling complex agonists[8] and adenosine A2A receptor antagonists[9] are gaining momentum in the PD research.

Number of studies reports evidence of link between brain renin-angiotensin system(RAS) and PD. Recent studies have also shown involvement of brain RAS with other neuronal conditions like stress and anxiety,[10] depression, cognitive dysfunction[11] and alcohol intake.[12] AT1 receptor inhibition has been shown to be associated with improved learning, spatial memory and motor co-ordination.[13,14]With deeper understanding of brainRAS and its interaction with hepatocyte growth factor (HGF) / c-Met receptor systems, newer targets of PD therapeutics have opened. Angiotensin I, II, III and IV are shown to influence the vasoconstriction, neuroinflammation, oxidative stress and apoptosis. They bring these effects through angiotensin 1-7 and angiotensin 3-7 subsidiaries acting via AT1 receptors. Conversely, the same angiotensin derivatives acting via AT2 and AT4 receptors are shown to produce angiogenesis and bring about anti-inflammatory, anti-oxidative and anti-apoptotic effects.[15]

With oxidative stress induced dopaminergic neuronal loss increasingly being implicated as one of the important aetiological factor, it is imperative to evaluate the level of free radical induced injury of the brain while evaluating the toxic rodent model.[16]The molecules that are modified by interactions with reactive oxygen species and those that change in response to increased redox state are the markers of oxidative stress. Reactive oxygen species can specifically alter the DNA, lipids and proteins in the brain. This can lead to detrimental effects from altered neuronal functions to the death of neurons.[17]

With this background, this study was designed to evaluate the antidepressant action and neuroprotective roles of captopril and perindopril in animal model of Parkinsonism. The objective of the present study was to evaluate the antidepressant properties and anti-oxidant properties of captopril and perindopril in paraquat model of Parkinsonism in mice.

MATERIALS AND METHODS

Animals and groups

Healthy adult albino mice of either sex weighing 100-150g were. All mice were obtained from animal house, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state & KMCH College of Pharmacy, Coimbatore, Tamil Nadu. Institutional animal ethics committee, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state, (with CPCSEA, India registered) (approval letter number: 33/16, dated-16.01.2016) and also Institutional animal ethics committee, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, (approval letter number: KMCRET/PhD/21/16-17, dated-22.02.2016) approved the study before the start of the study.

Induction of Parkinsonism - Paraquat model

The paraquat (Sigma chemicals, Mumbai) was stored according to the manufacturer label to prevent its decomposition. The Paraquat solution was freshly prepared at 7mg/kg. Paraquat injected intraperitoneally at the dose of 7mg/kg bodyweight at an interval of 2 days.

All the animals were monitored with a check list twice daily for the appearance of parkinsonian features, bradykinesia, postural instability, gait disturbance and rigidity.

Animals that died after the intraperitoneal injection were excluded from the analysis. Animals that failed to experience the parkinsonian symptoms were also excluded.

Animals that died within minutes of an injection and that did not experience parkinsonian symptoms were excluded from analysis.

Evaluation of depression influences

Depression influences on behaviour was studied with forced swim test and tail suspension test.

Forced swim test

Mice were placed in an open cylinder having a diameter of 10 cm and height of 25 cm with water up to 15 cm at 25 ± 1^0C. The mice were forced to swim in this small space. This induces a circumstance of immobility behaviour. When the animal ceases to struggle to get out and attains a state of floating with immobility, with minimal movements to keep the head above water, the time was measured. The total duration of the FST

was fixed at 240 seconds (4 minutes). The entire duration for each animal was video recorded and analysed later in a PC. During behavioural analysis, the time each mice spends mobile is measured and noted. The total mobility time is subtracted from 240 seconds. This represents the immobility time. This method was adopted as it is better to note the movements than the lack of movements. Any movement other than those necessary to balance to body and keep the head above the water was considered as mobility or movement.[18]

Tail suspension test: mice were gently lifted by their tails and slowly placed on a support. The grid was inverted resulting in mice to hang from the grid upside down. Care was taken to prevent it from falling down and getting injured by mounting the grid at 20 cm from the ground level. The grid was also provided with a 3 inch wall to prevent mice from traversing to the higher levels of the grid. The animals were made to stay on the grid for 240 seconds (4 minutes).[19] The tail hanging time was estimated in 10 chances with 1 minute interval between the trials. The immobility time was defined as time during which the animal was hanging passively. This was measure of depression.

Dissection of brain and processing of the two hemispheres

All the rats were anaesthetized using thiopental sodium (50 mg/kg) after 24 hours of behavioural monitoring. All micewere sacrificed by cervical decapitation. The brain was dissected out of the cranial cavity. Each brain was hemisectioned along the longitudinal fissure into right and left halves. One hemisection was homogenized and used for estimation of oxidative stress markers.

Enzymatic antioxidant activity
Estimation of reduced glutathione

To 250 µL of tissue homogenate taken in 2 ml eppendroff tube, 1 mL of 5% TCA was added and the above solution was centrifuged at 3000 g for 10 min at room temperature. To 250 µL of the above supernatant, 1.5 ml of 0.2 M phosphate buffer was added and mixed well. 250 µL of 0.6 mM of Ellman's reagent (DTNB solution) was added to the above mixture and the absorbance was measured at 412 nm within 10 min. A standard graph was plotted using glutathione reduced solution (1 mg/mL) and GSH content present in the tissue homogenates was calculated by interpolation. Amount of glutathione expressed as µg/mg protein. [20]

Lipid peroxidation assay

To 100 µL of the tissue homogenate, 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (TBA 0.37%, 0.25 N HCl and 15% TCA) was added and mixed. The above content was incubated in a boiling water bath for 15 min, cooled and centrifuged at 3500 rpm for 10 min at room temperature. The pink colour developed was estimated at 535 nm against a reagent blank, in a spectrophotometer. LPO was expressed as nmol of MDA/mg protein.[21]

Myeloperoxidase (MPO) activity estimation

MPO was measured with tetramethybenzidine method. To 80 µl 0.75 mM H_2O_2 and 110 µl TMB solution, 10 µl sample were added. The mixture was incubated at 37^0C for 5 minutes. 50 µl 2 M H_2SO_4 stopped the reaction and absorption was measured at 450 nm to measure MPO activity.[22]

Catalase activity

The homogenate mixture was mixed with 1.95 mL of 50nM phosphate buffer and 1 mL of 30mM hydrogen peroxide was added. The catalase activity was measured as 240nm at 15 seconds intervals. The catalase activity was estimated according to change in absorbance/minute of catalase with respect to extinction coefficient of

Table 1. Five groups (each having 6) of mice that were used in the study (n=30)

Groups	Group specifications
Group 1	Control group - Equivalent normal saline i.p
Group 2	Negative control: paraquat (25 mg/Kg BW i.p)
Group 3	Positive control: Levodopa (12 mg/Kg) and Benserazide (3 mg/Kg BW i.p) + paraquat (7 mg/Kg BW i.p)
Group 4	Captopril (20mg/kg BW i.p) + Paraquat (7 mg/Kg BW i.p)
Group 5	Perindopril (5mg/kg BW i.p) + Paraquat (7 mg/Kg BW i.p)

hydrogen peroxide (0.071 mmol cm-1). Catalase activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.[23]

Superoxide Dismutase (SOD) Level

0.1 mL of supernatant of homogenate mixture was mixed with 0.1 mL EDTA (1×10^{-4} M), 0.5 mL of carbonate buffer and 1 mL of epinephrine (1 mM). Spectrophotometric measurement of the mixture was measured at 480nm for 3 minutes. The SOD activity was expressed in terms of U/min/mg.[23]

Monoamine oxidase A and B (MAO – A and MAO – B) estimation:

The brain homogenate mixtures were added with 4 mM 5-HT and 2 mM b-PEA as specific substrates for MAO-A and MAO-B respectively. Final volume was made as 1 ml by adding 100 mM sodium phosphate buffer. The tubes were incubated at 37^0C for 20 minutes and stopped by adding 1 M HCl (200 ml). The products were extracted with 5 ml of butyl acetate (for MAO-A) and cyclohexane (for MAO-B) respectively. This

extract was measured at wavelength of 280 nm for MAO-A assay and 242 nm for MAO-B assay with spectrophotometer respectively.[24]

Statistical analysis

Data obtained from each model were tabulated separately and subjected to statistical analysis. Data were tabulated and presented as tables and diagrams. For all continuous parameters, mean ± standard deviation were calculated for each group. Comparison of the data was done by one way ANOVA test. All the parameters were compared with control group values. All calculations were done with software SPSS V 20 32bit. P value of less than 0.05 was taken as significant.

RESULTS

Two percent of the mice that were initially recruited died during induction of Parkinsonism and four percent of the mice died during subsequent evaluation of the properties of test drugs. Seven

Table 2. Tabulation of depression behaviour among
six groups (n=6) of mice of paraquat model

Group	Forced swim test (immobility time in sec)	Tail suspension test (immobility time in sec)
Control group	121 ± 4.9	149 ±4.2
Paraquat group	185.5± 5.2†	187.3 ± 4†
Paraquat +levodopa group	138.3 ± 3.7*	148.1 ± 3.4
Paraquat + Captopril group	83.6 ± 4.6†	87.5 ± 5.1†
Paraquat + perindopril group	87.3 ± 3.5†	94.1 ± 7.2†

Table 3. Tabulation of oxidative stress markers in paraquat model

	Control group	Paraquat group	Paraquat + levodopa group	Paraquat + captopril group	Paraquat + perindopril group
GSH	0.31±0.8	0.2±0.01†	0.26±0.01*	0.28±0.01	0.23±0.01*
LPO	11.8±2.7	21.5±3.4†	31.4±3.7†	21.8±3.5†	29.5±5.2†
MPO	59.8±2.5	107.2±1.81†	75.1±2.3†	90.7±9.6†	77.1±2.9†
Catalase	4.58 ± 0.07	2.12 ± 0.05†	4.25 ± 0.05	5.2 ± 0.09†	5.96±0.14†
SOD	2.54 ± 0.12	1.5 ± 0.01†	4.32 ± 0.04†	5.32±0.24†	4.85± 0.58†
MAO-A	0.8±0.03	0.77±0.05	0.74±0.02*	0.52±0.03†	0.79±0.02
MAO-B	1.1±0.05	1.19±0.01*	1.2±0.01*	1.2±0.05*	1.2±0.01*

Glutathione (GSH, oxidised/min/g protein), Lipid peroxidation (LPO, nmol of MDA/mg protein), myeloperoxidase (MPO, μmol/min/mg tissue), catalase (imoles of H_2O_2 used/min/mg protein), Superoxide dismutase (SOD, units/mg protein) and monoamine oxidase (MAO-A and MAO-B, u/mg protein) from each group (n=6) expressed as mean ± standard deviation, † *p<0.001, *p<0.05* as compared to control group

percent of the micewere excluded from the study because of insufficient induction of Parkinsonism with paraquat.

Evaluation of depression influences

Forced swim test

Paraquat induced mice had significant higher immobility time indicating the depressive behaviour after exposure to paraquat. The mice pre-treated with levodopa had more immobility time compared to control group. The test group mice exposed to captopril and perindopril had significantly less immobility time in comparison to the paraquat group, indicating anti-depressant effects of these drugs.

Tail suspension test

Paraquat induced mice had higher immobility time compared to the control group. There was no significant difference between immobility time of control and levodopa group. The mice pre-treated with the captopril and perindopril had lower immobility time in comparison to the paraquat group. The immobility time was significantly below the control group. This indicates effective anti-depressant action of these drugs in paraquat model.

Oxidative stress markers

• Glutathione levels were significantly decreased in paraquat only group, levodopa, and perindopril groups.

• Lipid peroxidation levels were significantly increased in both captopril and perindopril groups in comparison to the control group.

• Myeloperoxidase levels were significantly increased in both captopril and perindopril groups in comparison to the control group.

• Catalase levels were significantly increased in groups pre-treated with levodopa, captopril and perindopril.

• SOD levels were significantly increased in groups pre-treated with levodopa, captopril and perindopril. SOD levels were significantly lower in paraquat alone group in comparison to the control group.

• MAO-A levels were decreased in levodopa and captopril group

• MAO-B levels were increased in levodopa, captopril and perindopril group.

DISCUSSION

The most common psychiatric complication of PD is depression. Depression influences the quality of life in PD.[25] Nearly 40 - 50% of the patients with PD suffers from depression.[26] This indicates that depression has independent disease process rather than reaction to the disability and loss of independent function.[27] With the concomitant loss of noradrenergic and serotonergic neurons outside the substantia nigra of midbrain that occur in the later part of PD, these non-motor symptoms gains importance in the therapeutic management.

Most commonly, the behavioural despair is evaluated with forced swimming test. The immobile duration spent by the mice on the water is the measure of depression-like behaviour. Conventional and modified forms of this test are in vogue.[28] In the modified form of this test, activity scoring (swimming or climbing) provides a measure of rodent's response to drowning.[29] Many drugs have shown to improve the immobility duration spent in this test and it is successfully used to pre-clinically test antidepressant efficacy of these drugs. In the present study, both captopril and perindopril showed improved performance in forced swim test in paraquat model. Forced swimming test has been successfully used to evaluate the antidepressant activity of imipramine[30], fluoxetine, reboxetine, moclobemide[31] and nitroindazole.[32] It is proven that serotonin-selective reuptake inhibitors (fluoxetine, centraline, sertraline) increase swimming behaviour. Drugs that increase norepinephrine and dopamine improves climbing behaviour.[33] From this study, it is evident that captropril and perindopril improves immobility duration in forced swimming test by influencing serotonin levels, however, exact mechanism cannot be ascertained with this study design.

Role of oxidative stress in neuroinflammatory processes has been extensively evaluated with various drugs and plant products.[34] Most of the studies advocate a direct relationship of free radicals with microglia, astrocytes and neurons in this process.[35] In the present study, oxidized glutathione levels were significantly lower in mice pre-treated with captopril and

perindopril in comparison to paraquat group. The increased level of lipid peroxidase indicates effective and significant scavenging of free radical species during and after toxin induced neuronal damage in the test drug pre-treated animals.Similar neuroprotective roles of some of the herbal derivatives from ginger (curcumin), ginseng (ginsenoside) and polygonumcuspidatum (resveratrol) has been reported.[36] Herbal extracts like moutan cortex, Angelica dahurica root, and bupleurum root also exerts neuroprotective action in PD.[37] Many flavonoids are proposed to exhibit neuroprotective actions primarily through anti-oxidant mechanisms.[38] Apart from these, artemisia[39], isolongifolene[40] and caffeine[41] and many other drugs have shown significant therapeutic roles in PD.

Limitations of the study

In the forced swimming test for evaluation of antidepressant activity of the rodents, present study concentrates only on the immobility time. Evaluation of climbing efforts and swimming behaviour would have given deeper understanding of differential effects of serotonin, dopamine and norepinephrine.

CONCLUSION

Captopril and perindopril have shown beneficial effects for depression (as evidenced through forced swimming test and tail suspension test) in paraquat model of Parkinsonism. These drugs reduce the oxidative stress in paraquat mice model of Parkinsonism.

ACKNOWLEDGEMENTS

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