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Neurobehavioral Effects of Losartan on Rotenone Induced Parkinsonism in Rats

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Abstract

Background and Objectives: Reticular activating system is increasing implicated as new target for therapeutic approaches in Parkinsonism. The objective of the present study was to evaluate the neurobehavioral effects of losartan in rotenone rat model of Parkinson's disease.

Methodology: Adult Wistar albino rats were divided into four groups of six each. Parkinsonism was induced with rotenone (3 mg/Kg intraperitoneal) in three groups. One group (positive control) was treated with levodopa (12 mg/Kg) and Benserazide (3 mg/Kg). Other group (losartan group) was treated with losartan (90 mg/Kg). Motor functions were evaluated by rota rod test and spontaneous locomotor activity. Exploratory behaviour was evaluated by hole board test. Depression influences on behaviour was studied with forced swim test. Elevated plus maze test was used for analysing anxiety influences on behaviour.

Results: The mean time of losartan pre-treated rats that stayed in the accelerating rota rod was significantly shorter compared the control, rotenone and levodopa group. Locomotor activity as measured by actophotometer was significantly decreased in the losartan group rats. The time spent by losartan group rats as immobile in water was significantly higher than the other groups. Losartan group rats had significantly higher number of head dipping in the hole board test. There was no significant change in the open arm activity both in duration and in number of entries among rats treated with levodopa and losartan

Conclusion: Losartan improves neurobehavioral indicators in rotenone rat model of Parkinson's disease.

***Key words**: angiotensin receptor blocker; parkinsonism; renin angiotensin system; behavioural study*

Introduction

The progressive degeneration of dopaminergic neurons in substantia nigra pars compacta is the established neuronal change in the Parkinsonism. Neuroinflammatory processes accelerate the process of neuronal loss, oxidative injury resulting in alterations in mitochondrial membrane permeability, enzyme metabolism and mitochondrial genome changes.[1]the symptomatic triad of bradykinesia, tremors-at-rest, and rigidity occur. Progressive neurodegeneration may also impact non-DA neurotransmitter systems including cholinergic, noradrenergic, and serotonergic, often leading to the development of depression, sleep disturbances, dementia, and autonomic nervous system failure. L-DOPA is the most efficacious oral delivery treatment for controlling motor symptoms; however, this approach is ineffective regarding nonmotor symptoms. New treatment strategies are needed designed to provide

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neuroprotection and encourage neurogenesis and synaptogenesis to slow or reverse this disease process. The hepatocyte growth factor (HGF In addition to these mechanisms the brain renin angiotensin system (RAS) maintaining the body water balance, blood pressure, sexual behaviours and pituitary glandular secretions with influences in the learning and memory functions of the brain has been repeatedly established.[2] This brain RAS system plays a role in pathogenesis of certain neurodegenerative disorders like Alzheimer's disease[3] and Parkinson's disease.[4,5] Angiotensin II acts on the certain areas of the brain influencing the drinking behaviour and natriuresis.[6] It stimulates the vasopressin release, modulate the sympathetic outflow and decrease the baroreceptor reflex.[7] It is postulated that most of these effects are through AT1 receptors. Animal studies have added the necessary evidence to the notion that AT1 receptor influences the cell proliferation, water intake and blood pressure.[7,8] Angiotensin II can stimulate catecholamine release through AT1 receptor stimulation.[9] Rodent studies have also shown that angiotensin receptor binding in substantia nigra pars compacta and bring about presynaptic effects in the dopaminergic neurons in the region.[10–12]

Studies have shown that losartan, the antagonist of AT1 receptor, protects dopaminergic neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity both in primary ventral mesencephalic cultures as well as in the substantia nigra pars compacta of mice.[13] Few interventional trials in atypical Parkinsonism have also ascribed promising role for losartan in neuroprotection.[14] Effects of losartan on midbrain dopaminergic neurons against rotenone-induced cell death has been positive.[15] Another AT1 receptor blocker, candesartan, has shown promising results in rotenone rat model of Parkinson's disease.[16] However, the neuroprotective role of losartan in rotenone rat model has not been established.

The objective of the present study was to evaluate the neurobehavioral effects of losartan in rotenone rat model of Parkinson's disease.

Methodology

Animals and groups

Healthy adult Wistar albino rats of either sex weighing 180-250g were selected and divided into four groups. All rats 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.

Table 1: Four groups (each having 6) of rats that were used in the study (n=36).

Groups	Group specifications
Group 1	Control group - Equivalent normal saline i.p
Group 2	Negative control: Rotenone [3 mg/Kg BW i.p]
Group 3	Positive control: Levodopa [12 mg/Kg] and Benserazide [3 mg/Kg BW i.p]+ Rotenone [3 mg/Kg BW i.p]
Group 4	Losartan 90 mg/Kg BW i.p + Rotenone [3 mg/Kg BW i.p]

Parkinson's disease induction with end points

All rats were divided into four groups having six each (table 1). Three groups underwent Parkinsonsim induction with rotenone. The Rotenone (Sigma Chemicals, Mumbai) solution was freshly prepared at 3 mg/kg. The Rotenone was dissolved in dimethylsulfoxide and adjust to pH 7.4 with potassium hydroxide. Rotenone injected i.p at the dose of 3 mg/kg body weight, 7 days. The solution was used immediately after preparation as it was stable only for a period of 24 hours at 25^0C. All the rats 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.

Group 4 rats were given losartan (90mg/kg body weight) intraperitoneally for 7 days. All animals that did not survive 7 days of intraperitoneal injections were excluded from the study.

Behavioural Analysis

Motor functions were evaluated by rota rod test and spontaneous locomotor activity. Exploratory behaviour was evaluated by hole board test. Depression influences on behaviour was studied with forced swim test. Elevated plus maze test was used for analysing anxiety influences on behaviour.

Rotarod test[17] largely replacing rats as the behaviorist's animal of choice. Before aspects of behavior such as emotionality or cognition can be assessed, however, it is vital to determine whether the motor capabilities of e.g. a mutant or lesioned mouse allow such an assessment. Performance on a maze task requiring strength and coordination, such as the Morris water maze, might well be impaired in a mouse by motor, rather than cognitive, impairments, so it is essential to selectively dissect the latter from the former. For example, sensorimotor impairments caused by NMDA antagonists have been shown to impair water maze performance2. , Motor coordination has traditionally been assessed in mice and rats by the rotarod test, in which the animal is placed on a horizontal rod that rotates about its long axis; the animal must walk forwards to remain upright and not fall off. Both set speed and accelerating versions of the rotarod are available., The other three tests described in this article (horizontal bar, static rods and parallel bars: Motor co-ordination test was conducted by placing the rats on the horizontally placed rotating rod. The time taken by animals to fall from the rotating rod was noted. The length of time (duration) the animal stay on the rod without falling, gives a measure of their coordination, balance, physical condition and motor-planning.

Spontaneous locomotor activity[18]: Spontaneous horizontal activity was measured using actophotometer that operates on photoelectric cells connected with a counter. All the rats were placed individually in the activity cage for 3 min to habituate them before starting actual locomotor activity task for the next 3 min. the basal activity score was noted. Counts per ten min is used as an index of locomotor activity

Hole board test[19]: Rats were placed on a 25 cm elevated wooden board (40 cm X 40 cm) with 16 holes. A head dipping is counted when the animal introduces its head into any hole of the box up to the level of the ears. Decrease in anxiety shows increased exploration of the holes.

Forced swim test[20]: Rats were forced to swim in a glass cylinder (20 cm height, 14 cm diameter) containing 10 cm depth of water. Animal were considered to be immobile, when they made no further attempts to escape except the movements necessary to kept their heads above the water. The time spent by the rat as immobile in water represented the measure of depression – like behaviour.

Elevated plus maze test[21]: The rats were placed at the junction of the maze with four arms, facing an open arm. The duration in each arm were recorded by a video – tracking system and by an observer simultaneously for 5 minutes. The duration of the test period was 10 minutes, the number of entries into the open arm, number of entries into the closed arm, the time spent in open arm, and closed arm were noted.

Statistical analysis: The data obtained was expressed as mean ± standard deviation. Comparison of the data was done by one way ANOVA, followed by Dunnett comparison. P value of less than 0.05 was taken as significant.

Results

Table 2 shows the tabulation of all the parameters observed. Motor co-ordination and balancing was tested with rota rod test. The mean time of losartan pre-treated rats that stayed in the accelerating rota rod was significantly longer compared the control, rotenone and levodopa group. Particularly, the latency to the fall in losartan group was increased gradually as early as 7 days of losartan treatment. Locomotor activity as measured by actophotometer was significantly increased in the losartan group rats. The time spent by losartan group rats as immobile in water was significantly lower than the other groups. Indicating the drug used in the rats has taken away depression like behaviour. Losartan group rats had significantly higher number of head dipping in the hole board test, indicating the decrease in anxiety among these rats. There was no significant change in the open arm activity both in duration and in number of entries among rats treated with levodopa and losartan.

Table 2: Neurobehavioral effects of losartan among various groups of rats; mean ± standard deviation from each group of six rats each. *** p <0.001 and *p<0.01

Groups		Control group	Rotenone group	Rotenone + levodopa group	Rotenone + losartan group
Rota Rod test (in seconds)	Before	166.5 ± 3.4	172.5 ± 2	167.5±3.2	165.5±4.01
	After	164. 8 ± 4.5	103.67±8.20***	159.3±2.2	192.33±4.50***
Actophotometer (number of counts/10 min)	Before	502.3 ±19.3	494.3 ± 13.1***	444.5 ± 15*	410.5 ± 14***
	After	469 ± 8.1	184 ± 9	436.8 ± 18***	491.6 ± 14.8 ***
Forced swimming test (in seconds)	Before	149.3 ±2	151.8 ± 3.1	151.8 ± 3.5	149.1 ± 3.2
	After	151.6 ± 1	92.5 ± 4.6 ***	156.3 ± 2.1	81.3 ± 3.1***
Hole board test (number of times head dipping in the hole / 4 min)	Before	16.1 ± 1.5	16.1 ± 1.3	15.6 ± 0.5	14.2 ± 1
	After	14.6 ± 0.9	10.5 ± 0.6 ***	23.8 ± 1.2	19 ± 0.8 ***
Elevated plus maze test (number of entries in open arm)	Before	132.3 ± 5.8	121.1 ± 8	118 ± 3.1	120.1 ± 5.3
	After	114.3 ± 3.6	80.1 ± 3.6***	149.8 ±7.5	123.5 ± 3.1
Elevated plus maze test (time spent in open arm)	Before	183 ± 4.03	152.1±17.7	149.67±6.6	160.5 ± 11.6
	After	178.5 ± 11.4	124.3±3.3*	153.5 ± 3.1***	140 ± 3.4*

Discussion

Animal studies and clinical trials using losartan reported to have variable effects on dopaminergic function in substantia nigra pars compacta.[22]physiological and functional studies suggest that the brain renin-angiotensin system (RAS There is constant addition of evidence to establish a stronger association of brain RAS system with neurodegenerative disorders.[1,4,14,16,22]the symptomatic triad of bradykinesia, tremors-at-rest, and rigidity occur. Progressive neurodegeneration may also impact non-DA neurotransmitter systems including cholinergic, noradrenergic, and serotonergic, often leading to the development of depression, sleep disturbances, dementia, and autonomic nervous system failure. L-DOPA is the most efficacious oral delivery treatment for controlling motor symptoms; however, this approach is ineffective regarding nonmotor symptoms. New treatment strategies are needed designed to provide neuroprotection and encourage neurogenesis and synaptogenesis to slow or reverse this disease process. The hepatocyte growth factor (HGF On the contrary, a case report highlighted worsening of Parkinson symptoms with losartan.[23] However, the angiotensin receptor blockers are here to stay and continue to be experimented for the possible neuroprotective roles.

In the present study, the behavioural changes were observed even with a seven day administration of losartan to the rats that were induced with parkinsonism with rotenone. Rota rod rest and spontaneous locomotor activity evaluated motor functions. The losartan treated rats showed significant improvement in the motor functions. Similar improvement in the motor actions were noted in rats treated with Pseudoginsenoside in MPTP model.[24]

Hole board test evaluated exploratory behaviour in rats. Losartan treated rats had significantly higher head lowering exploratory activity indicating decrease in anxiety. Depression influences on behaviour was studied with forced swim test. Elevated plus maze test was used for analysing anxiety influences on behaviour. In the present study, the elevated plus maze test has no significant differences among rats treated with levodopa and losartan.

Overall, as there was significant improvement in the neurobehavioral activity among rats treated with losartan. Similar neuroprotective role is seen with candesartan[16] and Azilsartan.[25] Our study results are in line with many previous studies using captopril, perindopril, enalapril and moexipril in neuroprotective actions.[26]

Limitations of the study: administration of losartan for more duration would have resulted in more pronounced and equivocal neurobehavioral changes. A study design to note the specific nigrostriatal loss of neurons, either microscopic evaluation or molecular evaluation of apoptotic indicators would have resulted in unambiguous outcomes with regard to neuroprotection.

Conclusions

Losartan improves neurobehavioral indicators in rotenone rat model of Parkinson's disease

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Conflict of Interest: Authors declare no conflict of interest

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Ethical Clearance: 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.

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