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Original Research Article

Evaluation of locomotor activity of pioglitazone in albino mice

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ABSTRACT

Background: Pioglitazone (PIO), a Peroxisome Proliferator Activated Receptor γ (PPAR-γ) agonist, is an oral anti-diabetic agent belonging to the group of thiazolidinediones-TZDs used for the treatment of diabetes mellitus type 2 in monotherapy and in combination with a sulfonylurea, metformin, or insulin.
Methods: All animals were allowed to acclimatize with laboratory conditions at least two weeks before starting the experiment and they were maintained under the same condition throughout the experiment. They were given food and water ad libitum. The experiments were performed as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines. The animals were subjected to experimentation between 0900-1600 hours in noise free atmosphere with ambient temperature 23-300ºC.
Results: There was no significant reduction in the within group comparisons of the basal and final scores in locomotor activity.
Conclusions: The standard and test groups failed to produce any significant reduction in locomotor activity in the intergroup comparison as well as compared to normal control.

Keywords: Locomotor activity, Mice, Pioglitazone

INTRODUCTION

AD is a complex neurodegenerative disorder. The etiology of the disease has proven to be divergent with contributions from genetics, environment and normal aging process as well. There is ample evidence that multiple physiological functions are altered in AD. About 70% of the risk is believed to be genetic with many genes usually involved some of them being Apo lipoprotein E (ApoE), Amyloid Precursor Protein (APP), Presenilin 1 and Presenilin 2. Other risk factors include: history of head injuries, depression, hypertension, diabetes, high cholesterol and high homocysteine levels. Also thyroid disease, organic solvents, aluminium and herpes zoster infection have all been proposed, but the evidence is far from compelling.[1]

AD is characterized by dysfunctional intracellular and extracellular biochemical processes that result in neuron death.[2] It is associated with three pathological hallmarks: Loss of synapses and presence of neurofibrillary tangles and senile plaques.[3]

- Amyloid plaques, which are dense deposits of protein that accumulate outside and around nerve cells.
- Neurofibrillary tangles, which are twisted fibers that build up inside the nerve cell.

Elevated and long-lasting increases in cytoplasmic Ca^{2+} are consistent biomarkers of aging that represent one aspect of neuronal Ca^{2+} dyshomeostasis. Enhanced Ca^{2+} transients are also present in AD models and in cells from AD patients.[4] It is well documented that Ca^{2+}-mediated toxicity (excitotoxicity) occurs in response to prolonged

activation of NMDARs and that NMDAR antagonism provides neuroprotection in several culture models of aging and neurodegeneration, by reducing Ca^{2+} levels during an insult.

Current treatment includes Memantine, a noncompetitive antagonist of the NMDA-type glutamate receptor, Acetylcholinesterase (AChE) inhibitors like Tacrine, Donepezil, Rivastigmine and Galantamine and cognition enhancing (Nootropic) drugs like Piracetam. However, adverse cholinergic side effects in the periphery, narrow therapeutic range, and hepatotoxicity are among the several limitations to their therapeutic success.[5] Therefore, it is worthwhile to explore multi-potent agents aiming at diverse targets than the single target aiming counterparts in treating various cognitive disorders.

Piracetam reverses this effect in both rodents and human beings. Piracetam is a nootropic which improves the function of the neurotransmitter acetylcholine via muscarinic cholinergic (ACh) receptors, which are implicated in memory processes. Furthermore, Piracetam may have an effect on NMDA glutamate receptors, which are involved with learning and memory processes. It may exert its global effect on brain neurotransmission via modulation of ion channels (i.e., Na^+, K^+). It has been found to increase oxygen consumption in the brain, apparently in connection to ATP metabolism, and increases the activity of adenylate kinase in rat brains.

Pioglitazone (PIO), a Peroxisome Proliferator Activated Receptor γ (PPAR-γ) agonist, is an oral anti-diabetic agent belonging to the group of thiazolidinediones-TZDs used for the treatment of diabetes mellitus type 2 in monotherapy and in combination with a sulfonylurea, metformin, or insulin. Pioglitazone has also been used to treat non-alcoholic steatohepatitis (fatty liver), but this use is presently considered experimental. Pioglitazone has also been found to reduce the risk of conversion from pre-diabetes to diabetes mellitus type 2 by 72%. The neuroprotective effects of PIO against various brain insults have been rapidly accumulating. PIO has been found to have antioxidant, anti-inflammatory, antidepressant and anticonvulsant properties.

Based on evidence that Ca^{2+} dysregulation is a pathogenic factor of brain aging/AD, the impact of PIO on Ca^{2+}signaling/homeostasis in neurons has been tested and has been recently reported to have crucial roles in improving cognition and memory performance in early stages of AD.[6]

Scopolamine is a muscarinic receptor antagonist with amnestic properties that have been used for decades in experimental animals to induce impairment in their performance of a variety of tasks requiring intact working and reference memory. Scopolamine-induced memory impairment, particularly when coupled with a version of the inhibitory avoidance task provides a relatively rapid phenotypic screening tool for drug discovery in the field of cognition enhancement. The amnestic responses elicited by scopolamine in humans appear to mimic very closely the cognitive deficits associated with AD. Scopolamine induced memory impairment as a model system for AD is reversible, which lends itself well to screening methods in drug discovery.

METHODS

Adult Swiss albino mice (n= ninety-six) of either sex weighing between 25-35grams were used for this study. Albino mice were divided into 4 sets each consisting of 4 groups with 6 animals in each group. Mice were housed in groups of six in polypropylene cages with wood shavings as bedding, under controlled conditions of light at room temperature (25 ± 2^0C) and humidity of 55% under 12h light–dark cycle. All animals were allowed to acclimatize with laboratory conditions at least two weeks before starting the experiment and they were maintained under the same condition throughout the experiment. They were given food and water ad libitum. The experiments were performed as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines. The animals were subjected to experimentation between 0900-1600 hours in noise free atmosphere with ambient temperature 23-30^{0}C.

Animals used

- *Species:* Albino mice of Swiss strain.
- *Age (months):* Around 3-4 months.
- *Gender:* Both males and females.
- *Weight:* 25 -35 grams.
- *Number to be used:* Ninety-six.
- Number of days each animal was housed: one month.

Inclusion criteria

- Swiss Albino mice weighing 25 to 35g of either sex.
- Age 3-4 months.
- Animals acclimatized to experimental conditions for two weeks.
- Healthy mice with normal behavior and activity.

Exclusion criteria

- Mice weighing more than 35g and less than 25g.
- Diseased mice.
- Pregnant mice

Drugs and treatment

- Piracetam (Nootropil, UCB India PVT LTD, India.)
- Used as the standard drug at dose of 312mg/kg orally.
- Scopolamine hydrobromide trihydrate (Tokyo Chemical Industry CO., LTD. Tokyo, Japan)

Interoceptive model used to induce amnesia at a dose of 1mg/kg injected intra- peritoneal on the test day.

- Normal Saline

Used as vehicle (10ml/kg)

- Pioglitazone (PIOZ, USV LTD, India)

Used as the test drug at dose of 5.2mg/kg orally. Evaluation of Locomotor Activity Using Actophotometer[128].

Principle

Locomotor activity is considered as an index of alertness and decrease in it would indicate sedative activity. It is measured using an actophotometer.

Apparatus

It operates on photoelectric cells that are connected in the circuit with a counter. A photocell is activated when the rays of light falling on the photocells are cut off by animals crossing the beam of light. As the photocell is activated, a count is recorded. The photocells are connected to an electronic automatic counting device which counts the number of "cut offs."

Albino mice (25-35g) of either sex were divided into four groups with six mice in each group. Food was withheld for 12 hour prior to the test but water was supplied ad libitum. All groups were treated with respective drugs as mentioned in Table No 4. After 45 minutes of drug treatment, each mouse was placed individually in Actophotometer (DESH BIOLOGICAL WORKS, Ambala, India) for a period of 5 minutes and locomotor activity was measured in terms of scores. The percentage difference in the motor activity between basal (on Day 1) and final (on Day 8) scores, before and after drug treatment respectively, was calculated. The locomotor activity was expressed in terms of total photobeam count per 5 minute per mice.

RESULTS

The effect of Pioglitazone on locomotor activity was tested with actophotometer. This was done to evaluate any sedative or muscle relaxant property for the investigational drug. There was no significant reduction in the within group comparisons of the basal and final scores in locomotor activity. The standard and test groups failed to produce any significant reduction in locomotor activity in the intergroup comparison as well as compared to normal control.

The effect of PIO on locomotor activity was studied in mice using actophotometer and no significant alteration in motor activity was observed. For evaluating learning and memory function both Elevated Plus Maze (EPM), Passive Avoidance Paradigm (PAP) -step down method and Morris water maze (MWM) were used in mice after pharmacologically inducing amnesia using Scopolamine on the Test day (Day 7) and testing for retention 24 hours later. There were four groups with six animals in each group in all the three models comprising of Control (Normal saline 10ml/kg), Standard (Piracetam 312mg/kg), Negative control (Scopolamine 1 mg/kg), and Test (PIO 5.2mg/kg), that were evaluated for their potential to enhance cognition.

Table 1: Evaluation of locomotor activity of pioglitazone in mice: Basal and Final scores.

Groups (n= 6)		Treatment	Locomotor activity (LA) Scores per 5 min (Mean±SD)	
			Basal	Final
1	Control	Normal Saline	48.16±4.92 NS	49.17±5.6 NS
2	Negative control	Scopolamine	76.17±6.11 NS	79.83±6.68 NS
3	Standard	Piracetam	53.83±5.23 NS	51.17±6.43 NS
4	Test	Pioglitazone	57.67±4.89 NS	60.5±7.26 NS

Analyzed by Student's paired t- test for within group comparison and Dunnet's test for multigroup comparison.
[NS- not significant]

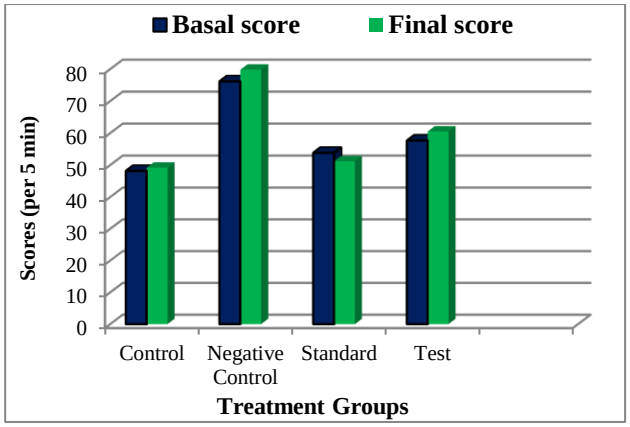
Table 2: Within group comparison of Basal scores (Day 1) and final scores (day 8) by student's paired t- test.

Groups	Mean difference	t- value	P- value
Control	-1.67	-3.95	0.11 NS
Negative control	-3.67	-1.79	0.13 NS
Standard	2.67	0.983	0.37 NS
Test	-2.83	-1.53	0.19 NS

P> 0.005- not significant- NS

The above table depicts comparison of basal score and final score on day 1 and day 8. Among controls the mean difference was -1.67 whereas among negative control it was -3.67 nevertheless it was not statistically significant.

Data represented as Mean±SD on day 1 and day 8 respectively (n=6). Analyzed by Oneway ANOVA followed by post hoc Dunnett's multiple comparison test (Figure 1).



Significance ($p < 0.05$)

Figure 1: Effect of pioglitazone on locomotor activity: basal and final scores (mean) on day 1 and day 8 respectively.

DISCUSSION

Dementia, a syndrome with many causes, refers to an acquired progressive deterioration in cognitive abilities severe enough to interfere with social, occupational and intellectual functions that impairs the successful performance of activities of daily living. Alzheimer's disease, the most common form of dementia, is a chronic, irreversible progressive neurodegenerative age-related brain disorder, characterized by fatal debilitating progressive memory loss and extensive deterioration of cognitive and functional abilities and behavioral disturbances. The pathological hallmarks of AD are Aβ plaques, NFTs, and neuronal loss.

The hippocampus, amygdala, cingulate gyrus and para hippocampal gyrus are the regions of the brain associated with learning and memory. In the early stages of AD, short-term memory begins to decline when the cells in the hippocampus degenerate. Amygdala is affected later than the hippocampus. As the disease progress, the cerebral cortex gets thinner, the brain gradually atrophies and the ventricles enlarge.

The cholinergic and glutamatergic neurotransmitter systems have been implicated in age-related cognitive decline, and neuroplasticity has been reported as a compensatory mechanism in response to Alzheimer's disease pathology.[7,8]

The management of AD is challenging and gratifying, despite the absence of a cure or a robust pharmacologic treatment. The primary focus of the currently available pharmacological agents (acetylcholinesterase inhibitors, and NMDAR antagonist) is on slowing the progression of symptoms, and on long-term amelioration of associated behavioral and neurologic problems to maximize the patient's ability to function in daily life and maintain the quality of life. They cannot stop or reverse the inexorable neurodegenerative process. And also, management of many of the non- cognitive psychiatric symptoms of AD often requires adjuvant treatment with antidepressants, antipsychotics, mood stabilizers and/ or hypnotics.[9] Given these limitations, it is therefore worthwhile to explore multi-potent agents aiming at diverse targets than the single target aiming counterparts in treating not only the symptoms, but also targeting the pathophysiology of AD.

Nootropics are a newer class of drugs with selective facilitatory effect on integrative functions of the CNS, particularly on intellectual performance, learning capacity and memory.[10] Nootropics, popularly referred to as "smart drugs or "memory/ cognitive enhancers", include Piracetam, Oxiracetam etc and also include supplements, functional foods or nutraceuticals that can enhance memory by increasing the levels of neurochemicals like neurotransmitters, neurohormones and enzymes, improving oxygen supply to the brain or by stimulating nerve growth.

Piracetam is shown to have documented clinical benefit in treatment of several conditions including age-related cognitive disorders, vertigo, cortical myoclonus, dyslexia and sickle cell anemia.

Many mechanisms are postulated for the nootropic activity of Piracetam. It influences the cholinergic function by promoting the uptake of choline and facilitates production and turnover of acetylcholine and produce the action at both muscarinic and nicotinic receptors. In human individuals, a drastic decline in Ach receptors is reported with ageing. Piracetam elevates the frontal cortex density of acetylcholine receptors by 30-40%, and also restoring the levels to those of healthy young mice.[11] Piracetam also elevates NMDAR density in the hippocampus by 20% and normalizes the enhanced affinity of L-glutamate for the NMDAR and also attenuates excess neuronal firing by reducing high voltage-dependent calcium influx into neurons. It also increases cerebral blood flow, cerebral oxygen usage, metabolic rate and cerebral glucose metabolic rates in chronic impaired human brain function conditions like multiinfarct dementia (due to stroke), senile dementia (Alzheimer type and pseudo dementia) and ischaemic cerebral infarcts. Even though, nootropics act on multiple targets, it is not a satisfactory remedy for age associated learning deficits and protection of degenerating neurons. Therefore, researchers are motivated to explore novel compounds and come up with a promising solution to manage neurodegenerative diseases.

Locomotor activity is considered as an index of motor activity and a decrease in it would indicate sedative action and motor incoordination. Pioglitzone was tested for any influence on locomotion using actophotometer and was found to have no effect on locomotor activity. A similar effect on locomotor activity of Pioglitazone was observed by Pathan et al.[12]

Learning and memory parameters were examined on different parameters on acquisition and retrieval data and were evaluated by using behavioral paradigms like Morris water maze, step-down passive avoidance methods and elevated plus maze.

CONCLUSION

The results from the present study demonstrates the cognitive enhancing role of PIO in presence of scopolamine induced amnesia, emphasizing its potential applications in neurodegenerative conditions associated with cognitive impairments like Alzheimer's disease.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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