Effect of Adenosine A2a Receptor Antagonist In Haloperidol Induced Model of Parkinson's Disease.

Syeda Najam Zehra
Hamdard University
Ishrat Younus (ishratyounas@gmail.com)
Hamdard University https://orcid.org/0000-0002-7032-6677
Saima Mahmood Malhi
Dow University of Health Sciences
Muhammad Liaquat Raza
Iqra University
Azfar Athar Ishaqui
Universiti Sains Malaysia
Sana Khan
University of Karachi

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Abstract

One of the major causes of neurological disorders is degeneration of neurons which is commonly termed as Neurodegeneration. It is well documented that two of the neurodegenerative disorders i.e., Alzheimer's Disease & Parkinson Disease comes in top fifteen causes of deaths in United States of America. Due to the neuroprotective property of adenosine A2a receptor antagonists (ZM241385) was evaluated in haloperidol mouse model of Parkinson's disease. Our results reveal significant antidopaminergic effects of adenosine A2A receptor antagonist that support its utilization as a new treatment approach in Parkinson's diseases.

Introduction

According to World Health Organization, hundreds of millions of peoples worldwide are currently effecting from different types of neurological disorders (Ekanem et al.; Siuly and Zhang, 2016). One of the major causes of neurological disorders is degeneration of neurons which is commonly termed as Neurodegeneration. Minino et al. (2013) reported that two of the neurodegenerative disorders i.e., Alzheimer's Disease & Parkinson Disease comes in top fifteen causes of deaths in United States of America (Miniño and Murphy, 2013). Some individual studies have reported a 0.3% prevalence of Parkinson Disease. This prevalence increases approximately up to 2% in persons with age above 65 to 85 years while prevalence of up to 5% was also reported in persons with age more than 85 years (Calabrese, 2007; Noyes et al., 2006; Weintraub et al., 2008).

Many of the symptoms are due to a loss of neurons that produce dopamine. When dopamine levels decrease, it causes abnormal brain activity, leading to impaired movement and other symptoms of Parkinson's disease. These clinical features include the motor symptoms of disease (include tremor rigidity, slowness of movement (Bradykinesia) whereas the non-motor symptoms that we identify today were known to a limited extent like disturbed sleep, depression, anxiety, constipation etc (Fahn, 2015; Jankovic, 2008; Lee and Trojanowski, 2006).

Adenosine is an important neuromodulator that helps in dopamine and other neurotransmitters responses coordination for different brain functions like normal signaling of neurons (Dunwiddie and Masino, 2001) motor function, memory, learning and mood (Shook and Jackson, 2011), sleep control (Reichert et al., 2016), function of astrocytes (Stockwell et al., 2017) and normal process of aging (Castillo et al., 2009). There are four different subtypes of adenosine receptors present in brain A1, A2A, A2B and A3 (Ishiwata et al., 2005). It is observed that A2a receptors are closely associated with D2 receptor of dopamine and both the receptors have opposite effects on each other's signaling in a way that inhibition of A2a receptors enhances the dopamine (D2 dependent) signaling and facilitate their responses whereas A2a receptors agonists inhibits signaling of D2 receptors (Fink et al., 1992; Schiffmann et al., 1991). The main cause of Parkinson disease is the loss of dopamine cells and their input in the striatum, in this way due to the close association of A2a receptors with dopamine these receptors could play beneficial role in the treatment of Parkinson disease (Chen et al., 2001). ZM241385

(Adenosine A2a receptor antagonist) is a selective and potent adenosine A2a receptor antagonist, previously has been tested for its antidepressant activity in mice (El Yacoubi et al., 2001), for the suppression of overactive bladder in 6-hydroxydopamine rat model of Parkinson disease (Kitta et al., 2012), in rat model of Parkinson disease for its Anti Parkinson's effect(Fathalla et al., 2016), and for its potential effects on amygdala kindle seizures and anticonvulsive effects (Li et al., 2012). Due to the neuroprotective property of adenosine A2a receptor antagonists ,the various compounds belonging to this class are in under high consideration for the neurodegenerative diseases like Parkinson's disease and Alzheimer's Disease (Dall'Igna et al., 2003). The aim of current study is to evaluate the effects of adenosine A2a receptor antagonist (ZM241385) in haloperidol mouse model of Parkinson's disease.

Methodology

Study Setting: The experimental lab was well ventilated with temperature maintained at ± 25°C and under 12 hour light and dark cycle.

Experimental Animals: 30 Male NMRI mice, 8–12 weeks old (weight: 20-23g) were utilized for this study. Animals were divided into different groups and housed in plastic cages with floor covered with bedding of hardwood shavings and ad libitum access to water and standard food.

Experimental Groups: Animals were divided randomly into 3 groups of six animals each as follows:

- Group 1: (Haloperidol Negative control Group) mice were treated with haloperidol 1mg/kg/day intraperitoneally for 7 consecutive days.
- Group 2: Animals were given haloperidol 1mg/kg/d (I/P) with half an hour prior treatment with test drug (ZM241385) with a dose of 3.5mg/kg/day (I/P) for 7 successive days.
- Group 3: Animals were given haloperidol 1mg/kg/d (I/P) with half an hour prior treatment with 5mg/kg/d of test drug (ZM241385) I/P for 7 consecutive days.
- For sub-acute toxicity testing of test material: Animals were treated with test drug 5mg/kg/d for 14 successive days.

Induction of Parkinson Disease with Haloperidol: Drug solution was prepared freshly daily by drawing 0.2ml of drug from the available haloperidol ampule (5mg/ml strength)which is then diluted in 0.8 ml of water for injection to make up the final concentration of 1mg/ml. Mice were treated with haloperidol 1mg/kg/day I/P for 7 successive days. Catalepsy induced by haloperidol was then evaluated by performing bar test and block test at 30, 90 and 120 minutes after drug administration.

Behavioral Tests: For behavioral analysis open-field test, elevated catalepsy test, and akinesia test were performed.

a) Open Field Test: Open field test is a frequently used behavioral test for measuring the rodent's general activity and exploratory behavior thoroughly. In order to determine the effect of Haloperidol and rotenone on locomotor activity, open field test was used.

Apparatus: For open field test standard cubic box was used measuring 72 x72 cm with walls of 50cm in height. The floor of the box was divided by the lines into 25 squares. Mice were assessed by placing individually in the middle of the box and observing them for 5 minutes.

During observation following behavioral parameters were noted

- No of squares crossed were counted
- Rearing: frequency of mice standing on hind legs were counted
- Duration of corner sitting: time (s) spent while sitting at the corner
- Duration of center sitting: time spent in center
- Locomotor activity: was then calculated by sum of square crossed and the rearing numbers before and after treatment.

b) Catalepsy: catalepsy can be defined as a condition in which animal is unable to correct its imposed awkward posture. In a normal condition animal will quickly regain its position whenever it is placed in an unusual or awkward position but in case of catalepsy animal will require longer period of time to correct the imposed posture (Sanberg et al., 1988).

Apparatus: In order to quantify catalepsy many tests have been developed over the past years like parallel bars, wire grids test, platforms or pegs etc. Among these the most commonly used is standard bar test (first introduced by Kuschinsky and Hornykiewicz (1972) (Banasikowski and Beninger, 2012; Costall et al., 1978). Here the animal is placed in an unusual position by putting their forepaws on a horizontal bar that is 6.5cm above from the base and the time taken by the animal to correct its position is noted which is called as index of intensity of catalepsy. The cutoff time was fixed at 3 minutes (180 seconds) for the test.

c) Akinesia test: Animals were simply placed on the flat surface i.e. table and the time to move all four of the limbs (latency) was noted in (Seconds) by using stopwatch. Cut off time here, was a One minute. The test was repeated daily at different time intervals of post injection. Normal control animal will move quickly while the diseased animal will find difficulty moving its all four limbs to move. Hence, the effect of test drugs can be screened easily (Mitra et al., 1992; Muralikrishnan and Mohanakumar, 1998; Weihmuller et al., 1989).

Result

Catalepsy Test:

Haloperidol 1mg/kg (Group-1) intraperitoneally induced significant catalepsy after half an hour of its administration with maximum effects achieved at 90minutes and last for almost 3-4hrs. (i.e. when the mice's forepaws were placed on horizontal bar their latency time was greatly increased as compared to the normal control group). Graph-1 represents comparison of catalepsy latency duration of Haloperidol negative control animals (Group 1) with animals that were given test drug- ZM241385 dose of 3.5mg/kg

(Group-2) and 5mg/kg (Group-3). The result of one-way ANOVA (Tuckey's post hoc) shows that catalepsy latency duration for Group-1 and Group-2 animals was found to be statistically significant ($p < 0.05$) less as compared to Group-3 animals.

Open Field Test:

Open field test results indicate Haloperidol treated mice showed notable decrease in the rearing behavior and locomotion as compare to the normal control group i.e., they prefer corner sitting in the field. Graph-2 shows the comparison of mean number of boxes crossed by Group-1 animals (Haloperidol Negative control animals) with animals that were given test drug- ZM241385 dose of 3.5mg/kg (Group-2) and 5mg/kg (Group-3). One way-ANOVA and Tukey's multiple comparison post hoc test shows that mean number of boxes crossed by Group-3 animals were statistically significant ($p < 0.05$) higher as compared to Group-1animals while mean number of boxes crossed by Group-2 animals were statistically similar ($p > 0.05$) to Group-1 animals.

Akinesia Test:

Graph-3 represents comparison of akinesia latency duration of Haloperidol Negative control animals (Group 1) with animals that were given test drug- ZM241385 dose of 3.5mg/kg (Group-2) and 5mg/kg (Group-3). One way-ANOVA and Tukey's multiple comparison post hoc test shows that akinesia latency duration for Group-3 animals was statistically significant ($p < 0.05$) higher as compared to Group-1 animals. Whereas, the akinesia latency duration for Group-2 was statistically non-significant ($p > 0.05$ i.e. statistically similar) for Group-1 animals.

Discussion

Parkinson's disease is a progressive neurodegenerative disorder with only symptomatic treatment available for it that too has limitation. The adverse central and peripheral reaction associated with the chronic administration of most competent drug levodopa/carbidopa and others dopamine replacement drugs, particularly in advanced stage of Parkinson disease, urges the researchers to identify and developed the alternative therapy i.e. non-dopaminergic drugs. In present study, our test compound ZM241385 (Adenosine A2a receptor antagonist) has showed significant anti-Parkinson activity in haloperidol induced mice models of Parkinson disease.

As, it is a well-known fact that the main motor symptoms of Parkinson disease is due to the depletion of striatal dopamine induced by the degeneration of dopaminergic (D2) neurons (Schapira et al., 2009). Numerous researches and investigation revealed the inverse interaction of these receptors with adenosine A2a receptors which privileged the role of A2a antagonists as a novel approach for Parkinson's management, moreover the expression of adenosine A2a receptors in basal ganglia is well recognized for the regulation of motor activity through different ways (Zheng et al., 2018), therefore from research point of view this non -dopaminergic class of drugs are in under high consideration as an anti-parkinson's agents (Fox et al., 2008; Oertel and Schulz, 2016; Richardson et al., 1997).

ZM241385 when administered at doses of 3.5mg/kg and 5mg/kg (I/P), it attenuates Parkinson disease symptoms as observed by improved results in open field test, Akinesia and Catalepsy bar test, however among these two doses 5mg/kg produces optimal increase in behavioral parameter like locomotion and rearing in open field test and decreased catalepsy latency time in bar test ($p < 0.05$) by antagonizing the A2a receptors at striatum, thus support it as a potential candidate for Parkinson's disease. Moreover, Post treatment daily observation of test drug i.e. ZM24138 5 shows no toxicity symptoms, all the animal were remain active and none of the animal was died, slight changes in the weight of the animals were observed but that too was statistically non-significant. Our results are also supporting the previous research done on this compound in rat.

In conclusion, the levodopa and other dopamine replacement drugs would remain the first choice in attenuating the P.D symptoms, however the development of dyskinesia and motor fluctuation associated with utilization of chronic dopamine therapy becomes a greater challenge. Hence divert the future research towards the identification and establishment of non-dopaminergic therapy that do not involve dopamine receptors and its enzymes. for e.g. adenosine A2A receptor antagonist as a new treatment approach because various preclinical research on different compounds belonging to this class provide sufficient evidence that supports the potential of this class in improving motor symptoms as well as decreasing the chances of dyskinesia and wearing off in P.D, therefore can be utilized as a novel approach for symptomatic management of both motor and some non-motor symptoms of parkinson's disease due to their neuroprotective property, however it requires proper clinical analysis to estimate the scope of this unique class in all stages of parkinson's disease.

Declarations

COMPLIANCE WITH ETHICAL STANDARDS

Ethical Approval: Ethical approval letter was obtained from Ethical Review Board of Hamdard University and the guidelines for animals were followed during experimental procedure.

Consent for publication: The consent for publication was taken from all co-authors.

Competing interests: Authors declare that they have no competing interest.

Financial disclosure: The authors have no financial relationships relevant to this article to disclose.

Data Availability: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS: I.Y. and M.L.R participated in planning and supervision of the study. S.N.Z. and S.M.M performed all experiments. S.N. assisted and helped in all experiments. A.A.I. participated in data analysis, and drafted the manuscript.

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Figures

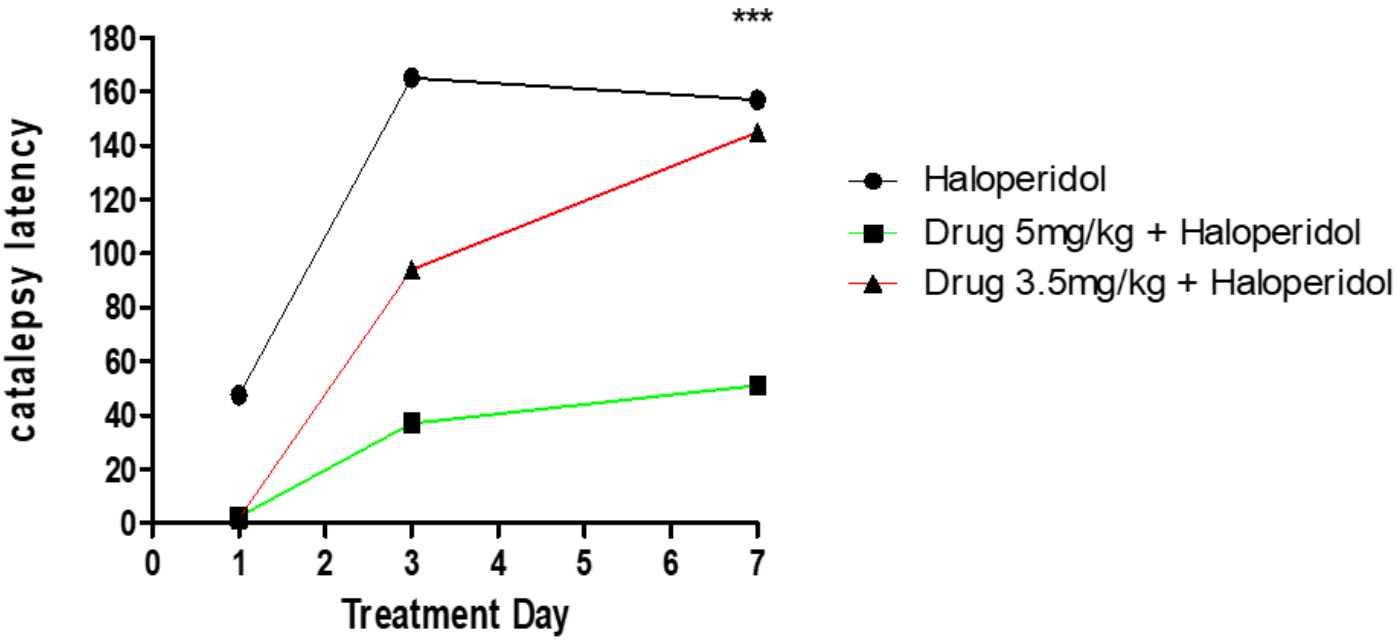

Figure 1

Comparison of catalepsy latency duration of Haloperidol & Test drug- ZM241385 (doses= 3.5mg/kg & 5mg/kg). Where; *** Represent p-value <0.001 = extremely significant

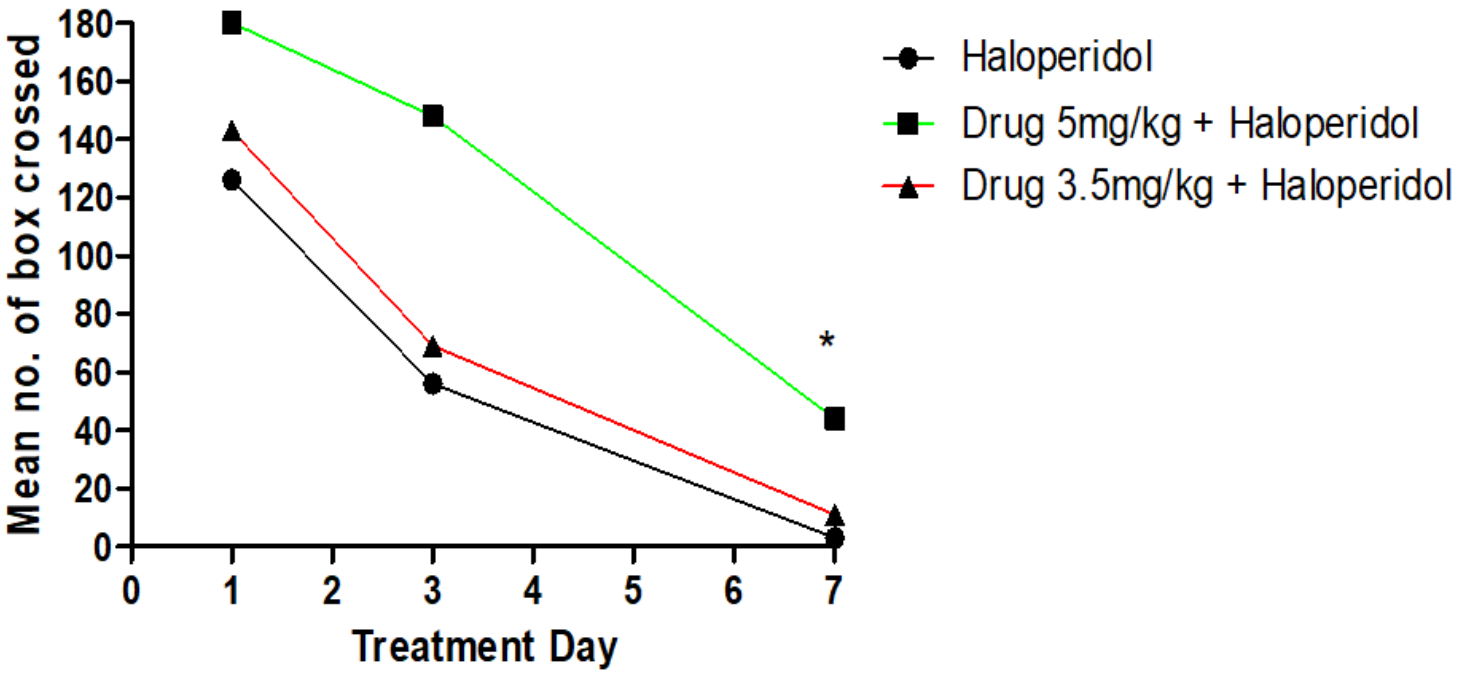

Figure 2

Comparison of Mean No. of Box crossed between Haloperidol Treatment & Test drug- ZM241385 (doses= 3.5mg/kg & 5mg/kg). Where; * Represent p- value 0.01 to 0.05= significant

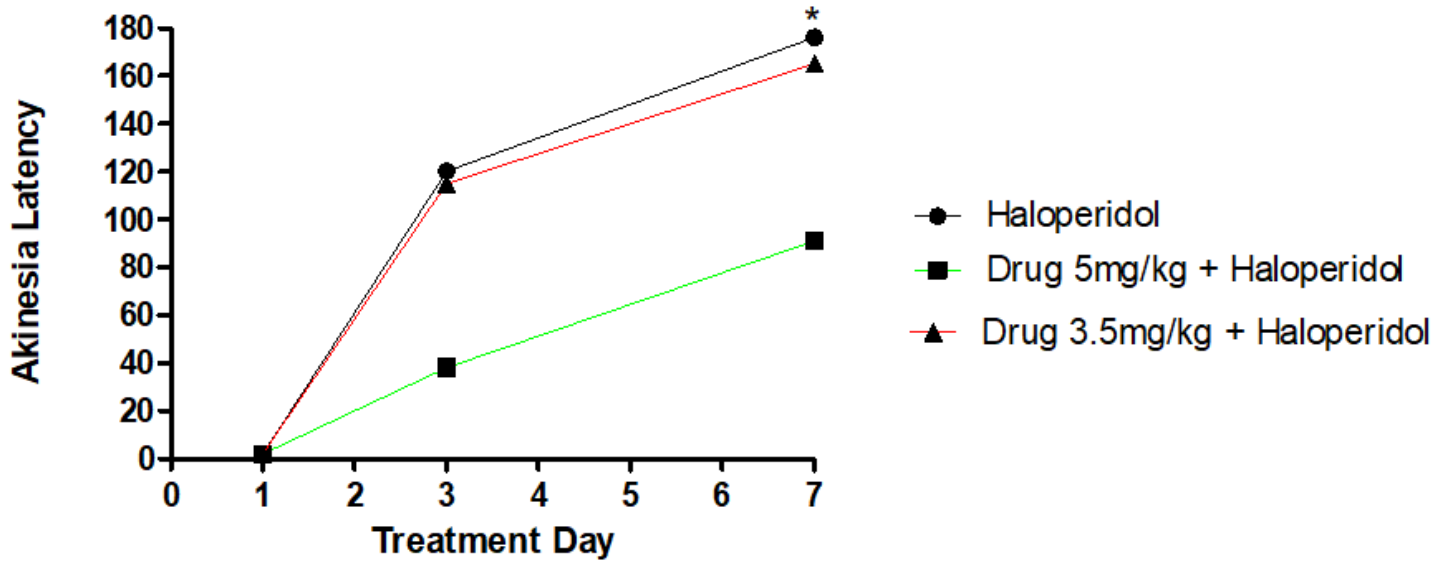

Figure 3

Comparison of Mean Akinesia latency duration of Haloperidol & Test drug- ZM241385 (doses= 3.5mg/kg & 5mg/kg). Where; * Represent p- value 0.01 to 0.05= significant