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An Experimental Study to Evaluate the Effect of Memantine in Animal Models of Anxiety in Swiss Albino Mice

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ABSTRACT

Background: Due to the adverse effects produced by the present conventional medicines for anxiety disorders, research for newer drugs is still desirable. From the literature it is evident that NMDA receptors play a key role in animal models of anxiety.

Aim: The present study is done to evaluate the antianxiety effect of memantine in swiss albino mice.

Materials and Methods: The experimental study was conducted from November 2014 to January 2015. Animals were divided into four groups. Twelve mice were randomly allotted in each group. Animals in the first group received normal saline as a control 10ml/kg, lorazepam 0.5mg/kg was administered to second group, memantine 3mg/kg as a test drug was given to the third group and memantine 3mg/kg + lorazepam 0.5mg/kg was administered to the fourth group. All the drugs were given for 7 consecutive days by intraperitoneal route.

Results: Results were analyzed by one-way ANOVA followed by Post-hoc Tukey's test. On the 1st day, memantine treated group did not show statistical significant anxiolytic effect in both the behavioural paradigms when compared to control group. On the 8th day, the animals showed significant decrease $p<0.001$ in step down latency period in shock free zone (185.4±3.87 Vs 278.3±5.49), significant increase $p<0.001$ in step down errors (6.8±0.78 Vs 1.4±0.19) and significant increase $p<0.001$ in total time spent in shock zone (32.1±2.22 Vs 5.6±0.6). In open field test, on 8th day the animals treated with memantine when compared to control group, showed significant increase $p<0.001$ in number of squares crossed (112.7± 2.69 Vs 83.2±2.96), time spent in central square (11.5±1.26 Vs 3.4±0.65), no. of rearings (32.4±2.61 Vs 17±1.81) and significant decrease $p<0.001$ in freezing time (15.2±1.12 Vs 20.2±2.29). Memantine showed synergistic antianxiety effect when combined with lorazepam.

Conclusion: Memantine showed significant anxiolytic effect in open field and passive avoidance response tests which are commonly used experimental models to assess anxiety states in animals.

Keywords: Anti-anxiety, Passive avoidance test, Open field test, Lorazepam

INTRODUCTION

The incidence of pathologic anxiety states is rapidly increasing among the community amounting to life time prevalence of 30.5% in women and 19.2% in males [1]. Hence, it is very important to address the problem of anxiety and explore safe, effective alternative medicines. The pathophysiology underlying the anxiety disorders is mostly associated with dysfunction of GABAergic neurotransmission. Although Benzodiazepines still remain the first line of anxiolytic drugs but side effects like sedation, addiction and development of tolerance may limit their chronic usage. However, it has been suggested that NMDA receptors may also contribute enormously to the neurobiological and psychological mechanisms in anxiety states [2-4].

In a previous study by Poleszak E et al., it was demonstrated that NMDA receptor activation antagonizes the NMDA antagonist-induced antianxiety effect in the elevated plus-maze test in mice [5]. This stimulated us to evaluate the anxiolytic effect of an uncompetitive NMDA antagonist, memantine which is currently used in Alzheimer's disease. Rationale for using memantine in our study is because of improved clinical tolerability of memantine when compared to other NMDA antagonists [6,7]. Phencyclidine, Dizocilpine which are other NMDA antagonists have demonstrated the antianxiety effects in rodents [8,9].

In our study, the test drug is memantine, which is a voltage and use-dependent blocker, can become trapped in the ligand-gated channel and has rapid blocking and unblocking dissociation kinetics. In a previous study by Shuijin He et al., [10], it was evident that chronic memantine administration to rat pups hippocampal slice cultures which served as differentiation-induced epileptogenesis model, increased the GABA release and $GABA_A$ receptor function. This also indicates the interplay of NMDA and $GABA_A$ receptors in anxiety states.

Earlier preclinical and clinical data have revealed the antianxiety effects of other competitive NMDA receptor antagonists and these classes of drugs are well compared to benzodiazepines or barbiturates as anxiolytics [11-13]. Previous studies undertaken by Bagewadi HG et al., has conclusively showed that memantine possessed the antidepressant effects [14,15], in animal models of depression and antianxiety effect [15], in Elevated plus maze model of anxiety. To strengthen our evidence, we carried out the present study to evaluate the antianxiety effects of memantine in different experimental models of anxiety like passive avoidance test and open field test.

The animal models of anxiety used today are mainly of two types. The first involves the animal's conditioned responses to aversive painful stimuli e.g. exposure to electric foot shock i.e. Vogel conflict test, four-plate test, passive avoidance response, defensive burying. The second is ethologically based paradigms and involves the animal's natural reactions like flight; avoidance and freezing that do not involve pain e.g. elevated plus maze, social interaction test, open field test, hole board test, staircase test [16]. In our study, we have used one conditioned and another ethologically based unconditioned model to avoid the bias and to assess the anxiolytic effect of memantine more effectively. Both open-field and passive avoidance tests are simple, having high sensitivity and specificity and are effective screening methods for different compounds to evaluate their anxiolytic activity [17].

In earlier study by Janel M Boyce-Rustay it was evident that NMDA receptor subunit (NR2A) knockout mice showed decreased anxiety-like behaviour in open field test when compared to wild-type mice

depicting the role of NMDA receptors in modulation of anxiety [18]. In the previous study done by Tomilenko RA [19], passive avoidance response test was used to evaluate the role of NMDA receptor agonist D-cycloserine and antagonist- Dizocilpine on learning and extinction of passive avoidance response in different anxiety states in mice.

The open-field and passive avoidance tests were used in earlier studies [17,20-26], to establish more conclusively the role of NMDA receptors in anxiety states. As our test drug is memantine- an uncompetitive NMDA antagonist and to make our study more appropriate, we have performed these two behavioural tests of anxiety. The BALB/C strains of swiss albino mice were used in our study. In the open field test, this strain of mice have reported with higher levels of anxiety-like behaviours when compared to C57BL/6 strain mice, which was consistent with previous findings by An XL [27].

The passive avoidance response test assesses anxiety states by training rodents to avoid electric shock stimuli by curbing their normal exploratory behaviour. In the previous study by Glotzbach et al., [28], it was evident that human participants in the Virtual reality (VR) arena, they gradually avoided the state which is associated with shock and exhibited greater amount of subjective fear when compared to participants who did not avoid the stimuli. Contextual fear conditioning predicts subsequent passive avoidance behaviour in a VR environment which is in homologous to passive avoidance response in animals. The pathological anxiety states in humans is usually associated with changes in physiological and behavioural responses to fear, painful stimuli and similar human responses were noted in animals to such stimuli. The open field and passive avoidance test simulate the human pathological anxiety state in rodents and they induce a fearful response by an aversive stimuli and preventing mice from their normal exploratory activity. This suggest the possibility of homologous, ethologically motivated defensive responses to such stimuli in animals and giving substantial face validity for both of these anxiety level assessment paradigms [29-34]. Thus, memantine was evaluated for its anti-anxiety effect in the present study.

MATERIALS AND METHODS

Swiss albino mice which belonged to either of the sex and weighed between 25 to 30 g were issued from animal house of MVJ medical college and research hospital. The animals were housed in polypropylene cages and were maintained in favorable conditions with a humidity 50-55% and temperature 22±2°C. CPCSEA guidelines were followed while maintaining and handling of the animals during the experimentation on the animals. Institutional Animal Ethics Committee permission was taken in order to carry out the study.

Drugs and Chemicals

Memantine was obtained from Sun Pharma drugs Pvt.Ltd. and lorazepam from Intas Pharmaceuticals Ltd. The drugs were diluted in normal saline and freshly prepared before drug administration.

Experimental Groups

The animals were divided into four groups. Twelve mice were randomly allotted in each group. Group I - was administered normal saline 10 ml/kg by intraperitoneal route (i.p.) which served as control group. Group II - was given memantine 3 mg/kg, i.p. Group III – received lorazepam 0.5 mg/kg, i.p. Group IV - was administered memantine 3 mg/kg, i.p. + lorazepam 0.5 mg/kg, i.p. All the animals in various groups received the respective drugs by intraperitoneal route for 7 days of experimental period. The experiments were carried out on the 1st day which was 24 hours after the 1st exposure of the animals to the open field and passive avoidance apparatus. The findings were again recorded on the 8th day. The anxiety behavioural assessment was performed on the days of testing i.e. on the 1st day and on the 8th day, 30 minutes after the drug administration. The dose of the memantine was chosen from previous studies done by Sufka KJ [35], and Fredriksson A [36], and dose of the lorazepam was chosen from earlier study by V. Sathyanathan [37].

Assessment of behavioural tests

Passive avoidance - The passive avoidance response was assessed in the apparatus with the dimension of 34 cm x 34 cm x 20 cm. In the center of the chamber a shock-free zone (SFZ) was placed. It consisted of grid floor through which 20 mV electric shock was delivered. Mouse was initially kept on the SFZ and electric shock was delivered whenever the mouse tried to come in contact with the grid floor. The animals by curbing their normal exploratory behaviour subsequently learned to avoid the aversive electric shock stimuli by remaining in the SFZ with freezing or minimal motility [38,39]. The mouse was trained for minimum of 60 seconds to stay at the SFZ. The following parameters were noted: 1.Step-down latency, the time interval during which the animal avoids electric shock and remain in the SFZ 2. Step-down error, number of times the animal tries to come back to SFZ in order to avoid painful electric stimuli. 3. Total duration of stay at Shock Zone (SZ).

Open Field test [17]- This is a standard behavioural model that assess anxiety states in rodents in which the anxious behaviour of mice to avoid open, unprotected area, preference for peripheral areas, along with periodic freezing were noted. A reduction in normal behaviours such as rearing and grooming were also considered as an index of anxiety. The apparatus consisted of floor space with dimension of 40cm x 40cm and 30cms in height. The floor space was divided into 16 squares equally. Prior to the testing, the mouse was placed at the center of the floor space and allowed to acclimatize with the surrounding area for 2 minutes. The following parameters were noted: 1) Duration for which animal stays in the central square; 2) Ambulation was indicated by the total no. of squares crossed; 3) Rearing was indicated by the total no. of times the animal stands on its rear paws.

STATISTICAL ANALYSIS

All the findings were expressed in terms of Mean± SEM. For comparison between the groups, one-way ANOVA followed by post-hoc Tukey's test was utilized. The p-value ≤0.05 was considered to represent statistical significant difference.

RESULTS

In passive avoidance test on the 1st day the animals treated with lorazepam as a standard, when compared to the control group, showed significant decrease p<0.001 in step down latency period in shock free zone (175.8±5.98 Vs 280.4±8.17), in step down error (6.4±0.82 Vs 1.5±0.29) and total time spent in shock zone (34.2±1.63 Vs 6.7±0.86) as shown in [Table/Fig-1]. On the 8th day, animals showed significant decrease p<0.001 in step down latency period in SFZ (161.4±2.73 Vs 278.3±5.49). The animals showed significant increase p<0.001 in step down error (8.1± 0.98 Vs 1.4±0.19) and total time spent in SZ (38.3±1.82 Vs 5.6±0.6) as shown in [Table/Fig-2].

In passive avoidance test, on the 1st day the animals treated with memantine when compared to control group, no statistical significant anxiolytic effect was as shown in [Table/Fig-1]. Whereas, on the 8th day the animals showed significant decrease p<0.001 in step down latency period in SFZ (185.4±3.87 Vs 278.3±5.49), significant increase p<0.001 in step down error (6.8±0.78 Vs 1.4±0.19) and in total time spent in SZ (32.1±2.22 Vs 5.6±0.6) as shown in [Table/Fig-2].

In passive avoidance test on the 1st day the animals treated with lorazepam when compared to memantine treated group, animals showed significant decrease p<0.001 in step down latency period

in SFZ (175.8±5.98 Vs 267.2±2.51), significant increase p<0.001 in step down error (6.4±0.82 Vs 2.1±0.64) and in total time spent in SZ (34.2±1.63 Vs 8.6±0.75) as shown in [Table/Fig-1]. But on the 8th day, there was significant decrease p<0.01 in step down latency period in SFZ (161.4±2.73 Vs 185.4±3.87) as shown in [Table/Fig-2]. On the 1st day, memantine + lorazepam treated group when compared to memantine alone treated group, animals showed significant decrease p<0.001 in step down latency period in SFZ (152.3±3.82 Vs 267.2±2.51), significant increase p<0.001 in step down error (9.4±0.56 Vs 2.1±0.64) and in total time spent in SZ (42.4±1.72 Vs 8.6±0.75) as shown in [Table/Fig-1]. But on the 8th day, animals showed significant decrease p<0.001 in step down latency period in SFZ (155.8±1.67 Vs 185.4±3.87), significant increase p<0.01 in step down error (9.2±0.63 Vs 6.8±0.78) and total time spent in SZ (41.8±1.81 Vs 32.1±2.22) as shown in [Table/Fig-2].

In open field test on the 1st day the animals treated with lorazepam as a standard when compared to control group, showed statistically significant anxiolytic activity as shown in [Table/Fig-3]. On the 8th day, the animals showed significant increase p<0.001 in no. of squares crossed (126.4±2.77 Vs 83.2±2.96), time spent in central square (14.3±1.53 Vs 3.4±0.65), no. of rearings (37.5±2.42 Vs 17±1.81) and significant decrease p<0.001 in freezing time (8.53±0.59 Vs 20.2±2.29) as shown in [Table/Fig-4]. In open field test on the 1st day the animals treated with memantine when compared to control group, no statistical significant anxiolytic effect was as shown in [Table/Fig-3]. Whereas on the 8th day animals showed significant increase p<0.001 in no. of squares crossed (112.7±2.69 Vs 83.2±2.96), time spent in central square (11.5±1.26 Vs 3.4±0.65), no. of rearings (32.4±2.61 Vs 17±1.81) and significant decrease (p<0.001) in freezing time (15.2±1.12 Vs 20.2±2.29) as shown in [Table/Fig-4].

Groups, (dose)	Step down Latency (s)	Step down Error (no.)	Time in shock zone (s)
1. Control (10 ml/kg, i.p.)	280.4±8.17	1.5± 0.29	6.7±0.86
2. Lorazepam (0.5mg/kg, i.p.)	175.8±5.98*	6.4± 0.82*	34.2±1.63*
3. Memantine (3mg/kg, i.p.)	267.2±2.51††	2.1± 0.64††	8.6±0.75††
4. Memantine + Lorazepam	152.3±3.82‡‡	9.4±0.56‡‡	42.4±1.72‡‡

[Table/Fig-1]: Effect of single dose observation in passive avoidance test on 1st day
(n=12), s-seconds, values expressed as mean±SEM. (*p< 0.001 vs. normal saline-control), (†p< 0.01, ††p< 0.001 vs. lorazepam), (‡p< 0.01, ‡‡p< 0.001 vs. memantine)

Groups, (dose)	Step down Latency (s)	Step down Error (no.)	Time in shock zone (s)
1. Control (10 ml/kg, i.p.)	278.3±5.49	1.4±0.19	5.6±0.6
2. Lorazepam (0.5 mg/kg, i.p.)	161.4±2.73*	8.1±0.98*	38.3±1.82*
3. Memantine (3mg/kg, i.p.)	185.4±3.87*,†	6.8±0.78*	32.1±2.22*
4. Memantine + Lorazepam	155.8±1.67‡‡	9.2±0.63‡	41.8 ±1.81‡

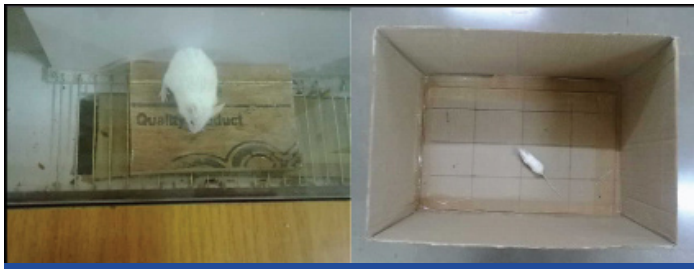
[Table/Fig-2]: Effect of multiple dose observation in passive avoidance test on 8th day.
(n=12), values expressed as mean±SEM. (*p< 0.001 vs. normal saline-control), (†p< 0.01, ††p< 0.001 vs. lorazepam), (‡p< 0.01, ‡‡p< 0.001 vs. memantine)

Groups, (dose)	Squares Crossed(no.)	Time spent in CS (s)	Rearing (no.)	Freezing time (s)
1. Control (10ml/kg, i.p)	86.3±1.08	2.7±0.14	19.3±1.6	20.6±2.36
2. Lorazepam (0.5mg/kg, i.p)	104.6±0.92*	8.4 ±0.18*	34.4±1.17*	9.6±1.35*
3. Memantine (3mg/kg, i.p.)	90.2±1.61††	3.1± 0.15††	23.5±2.39††	18.1±1.18†
4. Memantine + Lorazepam	135.6±1.6‡‡	26.32±1.1‡‡	51.2±2.06‡‡	7.3±0.86‡‡

[Table/Fig-3]: Effect of single dose observation in open field test on 1st day
s- seconds, CS-Central Square, (n=12), values expressed as mean±SEM. (*p< 0.001 vs. normal saline-control),(†p< 0.01, ††p< 0.001 vs. lorazepam), (‡‡p< 0.001 vs. memantine)

Groups, (dose)	Squares Crossed(no.)	Time spent in CS (s)	Rearing (no.)	Freezing time (s)
1. Control (10ml/kg, i.p)	83.2±2.96	3.4±0.65	17±1.81	20.2±2.29
2. Lorazepam (0.5mg/kg, i.p.)	126.4±2.77*	14.3±1.53*	37.5±2.42*	8.53±0.59*
3. Memantine (3mg/kg, i.p.)	112.7±2.69*,†	11.5±1.26*	32.4±2.61*	15.2±1.12*,†
4. Memantine + Lorazepam	131.2±2.98‡‡	25.2±1.17‡‡	46.1±1.51‡‡	5.9±0.62‡‡

[Table/Fig-4]: Effect of multiple dose observation in open field test on 8th day
s- seconds, CS-Central Square, (n=12), values expressed as mean±SEM. (*p< 0.001 vs. normal saline-control), (†p< 0.01, ††p< 0.001 vs. lorazepam), (‡‡p< 0.001 vs. memantine)



[Table/Fig-5]: Showing the mouse staying at shock free zone in passive avoidance test
[Table/Fig-6]: Showing the mouse crossing the central square in open field test

In open field test on 1st day the animals treated with lorazepam when compared to memantine group, animals showed significant increase p<0.001 in no. of squares crossed (104.6±0.92 Vs 90.2±1.61), time spent in central square (8.4±0.18 Vs 3.1±0.15), no. of rearings (34.4±1.17 Vs 23.5±2.39) and significant decrease p<0.01 in freezing time (9.6±1.35 Vs 18.1±1.18) as shown in [Table/Fig-3]. But on the 8th day there was significant increase p<0.01 in no. of squares crossed (126.4±2.77 Vs 112.7±2.69) and significant decrease p<0.01 in freezing time (8.53±0.59 Vs 15.2±1.12) as shown in [Table/Fig-4].

In open field test on 1st day the animals treated with memantine + lorazepam when compared to memantine alone group, showed statistical significant anxiolytic activity as shown in [Table/Fig-3]. On the 8th day, animals showed significant increase p<0.001 in no. of squares crossed (131.2±2.98 Vs 112.7±2.69), time spent in central square (25.2±1.17 Vs 11.5±1.26) no. of rearings (46.1±1.51 Vs 32.4±2.61) and significant decrease (p<0.001) in freezing time (5.9±0.62 Vs 15.2±1.12) as shown in [Table/Fig-4]. Photograph shows the mouse staying at Shock Free Zone (SFZ) in passive avoidance test shown in [Table/Fig-5]. Photograph shows the mouse crossing the central square in open field test as shown in [Table/Fig-6].

DISCUSSION

In the management of anxiety disorders, Benzodiazepines are mainly preferred as the first line treatment. However, chronic administration of Benzodiazepines results in side effects like sedation, amnesia, tolerance which limits their usage [40]. Mammalian brain mainly contains three types of inotropic glutamate receptors: N-methyl-D-aspartate (NMDA), 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate [41]. Among these, NMDA receptor plays a key role in modulation of anxiety disorders [42,43]. Interestingly it was found that when NMDA receptor antagonists were administered by microinjection into the dorsolateral periaqueductal gray area in rats, anxiolytic-like effects were more evident [44]. It was demonstrated from a previous study that in hippocampal neurons, conditioning with 20 μM NMDA for 20 sec caused 50% suppression of GABA responses and lorazepam potentiation reliably increased with $GABA_A$ receptors when there was NMDA-induced suppression in plasticity of fast synaptic transmission [45].

In passive avoidance test, the mice stay at the shock free zone in order to escape from the aversive electric shock stimuli. The reduction in normal behaviour is exhibited by decrease in step

down latency period and increase in number of step-down errors. Memantine showed statistically significant antianxiety effects in both the anxiety behaviour paradigms on the 8th day when compared to 1st day as shown in [Table/Fig-1-4]. Our study also demonstrated synergistic interaction between memantine and lorazepam in their antianxiety activity. Antianxiety effects of other competitive NMDA receptor antagonists were observed from previous studies [46,47].

Phencyclidine and its derivatives have produced anxiolytic effects in rodents via modulating NMDA, nicotinic acetylcholine and 5-HT receptors [46]. NMDA antagonist like phencyclidine which showed antianxiety effects in rodents causes characteristic side effects like hallucinations which limit its usage and ketamine produces profound drowsiness [47].

Further studies are needed to explore the antianxiety effect of memantine in other experimental animal models of anxiety like stair case test, vogel conflict test, social interaction test, novelty induced suppressed feeling latency test and hole board test to strengthen the evidence and address the anxiety disorders in the community in future. Recently, the over activity of hypothalamus pituitary adrenal axis (HPA) is postulated to play a significant role in anxiety disorders. The activity of the HPA axis is governed by the amygdala and hippocampus with interplay of various neuropeptides such as corticotrophin-releasing factor, substance P, vasopressin and neuropeptide Y (NPY) [48].

The hypothesis by Olney [49], suggest that over activation of NMDA receptors may cause subsequent damage of GABA neurons and further damage can be produced by disinhibited neurons e.g., glutamate, Ach, NPY. From the previous study by Wieronska JM et al., [50], it was evident that in the amygdala, the glutaminergic neurotransmission is mediated by NMDA receptors which may regulate neuropeptide Y neurons. Activity of memantine on NPY activity which might contribute to the antianxiety activity, throws light on its further exploration as antianxiety drug.

CONCLUSION

Memantine could be producing its antianxiety activity by blocking NMDA receptor. However, its modulating effect on NPY which might contribute to antianxiety effect cannot be ruled out. Further research is required to gain closer insights into the exact mechanism of action of memantine as antianxiety drug, which might benefit the patients of anxiety in clinical scenario.

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