Modulation of muscarinic system with serotonin-norepinephrine reuptake inhibitor antidepressant attenuates depression in mice

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ABSTRACT

Objective: Several studies suggest that muscarinic receptor antagonist scopolamine is a rapidly acting antidepressant for the treatment-resistant depression. Therefore, this study was carried out to investigate the possibility of synergistic potential of scopolamine with antidepressants for the treatment of depression without memory impairment in mice.

Materials and Methods: Antidepressants such as citalopram, duloxetine, fluvoxamine, and venlafaxine at their median effective dose that is 12.5, 42.8, 17.5, 15.7 mg/kg p.o., respectively, were evaluated in combination with scopolamine 0.2 mg/kg intraperitoneally for the synergistic potential for ameliorating depression in Swiss albino mice. A battery of tests including forced swim test (FST) and tail suspension test (TST) were performed in all the groups comprising vehicle control, scopolamine, antidepressants per se, and the combinations of antidepressants with scopolamine. This was followed by the locomotor activity and memory tests.

Results: Behavioral studies indicated that only antidepressant venlafaxine with scopolamine resulted in 95.5% and 93.6% reduction in immobility time compared to the vehicle control in FST and TST, respectively. This is significant (P < 0.0001) synergistic hyper-additive antidepressive-like effect compared to scopolamine per se and venlafaxine per se treatment effects in antidepressant paradigms. All the data were evaluated using the one-way analysis of variance followed by individual comparisons using Tukey’s post-hoc test. Control open field studies demonstrated no significant increase in general locomotion after co-administration of the compounds. Step down avoidance paradigm confirmed that scopolamine at the selected dose has no cognition deficit in any mice.

Conclusions: The dose of scopolamine selected for synergistic potential has no detrimental effect on memory. The present results suggest the concoction of scopolamine with venlafaxine for enhanced synergistic antidepressive effects with the reduction of dose.

KEY WORDS: Antidepressants, citalopram, duloxetine, fluvoxamine, forced swim test, scopolamine, step-down avoidance paradigm, tail suspension test, venlafaxine
Introduction

Depression is a serious disorder which, according to the World Health Organization, is one of the most prevalent and expensive psychiatric disorders of the developed world with a lifetime prevalence of 7.5% and 17%. Moreover, antidepressants are fraught with the problem of slow onset of action, treatment resistance, and patients show just 40% remission with the antidepressant drug therapy, according to the Sequenced Treatment Alternatives to Relieve Depression study.[1-2] Thus, the problems for antidepressant-resistant depressive patients still need to be solved,[3] and there is a need for effective and fast-acting antidepressants. Among the various treatment strategies, augmentation of the antidepressant potency is the most useful as it produces a synergistic effect with fewer side-effects. The previous literature survey supports this concept for the augmentation of antidepressant effects. Numerous studies reported N-methyl-D-aspartate (NMDA) receptor antagonist in combination with antidepressants for synergistic potential.[4-6] However, most of NMDA antagonist shows intrinsic neurotoxicity and adverse neurobehavioral side-effects.[7]

Antidepressant action has been observed with scopolamine similar to NMDA antagonist.[8,9] According to these studies, scopolamine produced robust and very fast onset of action clinically by changing the gene expression and synaptic plasticity. Witkin et al.[10] reported that muscarinic M1 and M2 receptors as potential future targets for the antidepressant drug strategy. However, scopolamine, which is muscarinic M1 and M2 receptor antagonist may have a cognitive deficit as side-effects.[8]

Thus, the present study was carried using antidepressants such as citalopram, fluvoxamine, duloxetine, venlafaxine in combination with scopolamine to study the synergistic effect of combinations in mice. This study aims to discover a unique combination which has a sub-therapeutic dose of antidepressants (median effective dose [ED50] doses) with a low dose of scopolamine for ameliorating depression without any cognition deficit in mice.

Materials and Methods

Animals

The study was carried out on Swiss albino mice, obtained from the breeder, Central Research Institute, Kasauli. Swiss albino mice weighing (20–25 g) were housed in the groups of 6 mice/cage in standard cages in a room temperature of 22 ± 2°C, under natural light/dark cycle and had free access to water and food (standard laboratory pellets) before the experiments. The mice were acclimatized at lab conditions for 5 days before the start of the experiment. All the experimental work had been carried out from 9:00 to 16:00. Antidepressant drugs were dissolved in distilled water and were given orally in conjugation with scopolamine. Scopolamine was also dissolved in distilled water and injected intraperitoneally (i.p.). All the drugs were given in a volume of 10 mL/kg. The experimental protocol number Institutional Animal Ethics Committee (IAEC)/consolidation and containerization point/14/PR-011 was duly approved by the IAEC and care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. 1181/ab/08/CPCSEA).

Drugs

Scopolamine, citalopram, duloxetine, fluvoxamine, and venlafaxine purchased from Sigma-Aldrich were used in the present study. ED50 doses of different antidepressants were first calculated in forced swim test (FST) using dose response curve with doses in geometrical progression versus immobility time in seconds. ED50 doses were then used in combination with scopolamine to study for synergistic potential. The dose of scopolamine 0.2 mg/kg i.p. was selected based on previous research done on scopolamine.[10] According to Ji and Zhang,[11] scopolamine at this dose, significantly decreased the immobility time (P < 0.001) in FST, but had no influence on the locomotor activity in open field test at this dose. Animals were randomized on the basis of their body weight into different groups such as vehicle p.o. (Group 1), scopolamine i.p. 0.2 mg/kg (Group 2), citalopram p.o. 12.5 mg/kg (Group 3), citalopram p.o. 12.5 mg/kg + scopolamine i.p. 0.2 mg/kg (Group 4), duloxetine p.o. 42.8 mg/kg (Group 5), duloxetine p.o. 42.8 mg/kg + scopolamine i.p. 0.2 mg/kg (Group 6), fluvoxamine p.o. 17.5 mg/kg (Group 7), fluvoxamine p.o. 17.5 mg/kg + scopolamine i.p. 0.2 mg/kg (Group 8), venlafaxine p.o. 15.7 mg/kg (Group 9), venlafaxine p.o. 15.7 mg/kg + scopolamine i.p. 0.2 mg/kg (Group 10). Figure 1 explains the general temporal sequence for the conduct of tests in the present study.

Procedure for Evaluation of Depression in Mice

Forced swim test in mice

Porsolt’s FST was used as a model for evaluating depression in mice.[12] Drugs were administered 30 min prior to the start of experimentation. Animals were individually forced to swim inside a glass jar containing 10 cm of water maintained at 23–25°C. After the initial 1–2 min of vigorous activity, the animals showed periods of immobility by floating with minimum movements. Animal were considered to be immobile, whenever it remained floating passively in the water in a slightly hunched but upright position, its nose above the water surface. The total...
immobility time for the period of 6 min will be recorded. The immobility time was recorded by an observer who was blind to the drug treatment.

**Tail suspension test in mice**

The total duration of immobility time was also checked by the tail suspension test (TST) according to the method described as a means of evaluating potential antidepressants with slight modifications. Treatment was administered 30 min before the test, and then the mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. A test was conducted in 6 min and immobility time was calculated. Mice were considered immobile only if they hung passively and completely without any body movements.

**Step down avoidance paradigm in mice**

Step down behavior was employed to examine memory loss by the procedure as described by Vignisse et al. with slight modifications. The apparatus (Digital: Rolex, Ambala) consisted of a plexiglass box with a grid floor, having a shock free zone (SFZ) (central platform) on the center of the grid floor. Electric shock (20 V AC) was delivered to the grid floor. One day prior to conducting the test, each mouse was trained to stay on the SFZ for at least 90 s; for this the animals were applied shock for 15 s every time when the mouse stepped down placing all paws on the grid floor. The process was repeated until the animal learned to stay on the SFZ for at least 90 s. Retention of memory was tested on the consecutive days by administrating treatment 30 min before the test. After the drug administration, each mouse was again placed on the SFZ, and then step down latency (SDL) and number of mistakes were observed for 5 min. A significantly increased SDL and decreased the number of mistakes compared to the vehicle control are the index of protective effect on the retrieval of memory.

**Locomotor activity test**

The Photoactometer (Digital: Rolex, Ambala) consisted of the square arena (30 * 30 * 25) with wire mesh bottom was used in this study. Six lights and six photocells were placed on the outer periphery of the bottom in such a way that the single mouse can block only one beam. Technically, its principle is that a photocell is activated when rays of light falling on the photocells are cut off by animals when crossing the beam of light. As the photocell activated count is recorded. The photocells are connected to an electronic automated counting device, which counts the number of “cut-offs.” These cut-offs were counted for a period of 10 min, and the figure was considered as a measure of the locomotor activity of the animal.

**Statistical Analysis**

The data were evaluated by one-way analysis of variance followed by individual comparisons using Tukey’s post-hoc test. All results are shown as mean ± standard error of the mean. ED₅₀ was calculated using GraphPad Prism 6 Software developed by GraphPad Software, Inc., USA.

**Results**

**Effects of Various Treatments on Forced Swimming Test in Mice**

The ED₅₀ values of different antidepressants obtained from the FST were citalopram 12.5 mg/kg p.o., duloxetine 42.8 mg/kg p.o., fluvoxamine 17.5 mg/kg p.o., venlafaxine 15.7 mg/kg p.o. in mice [Table 1].

Treatment with pharmacological interventions rejected the null hypothesis in FST and there was a difference observed within the mean values of different treatments (SS-15916, DF-8, MS-1990, F (8, 45) = 13.87, P < 0.0001). Scopolamine, citalopram, duloxetine, fluvoxamine, venlafaxine, citalopram with scopolamine, duloxetine with scopolamine, fluvoxamine with scopolamine, and venlafaxine with scopolamine decreased the immobility time 37%, 45.3%, 56.5%, 42.8%, 36.3%, 61.1%, 55.8%, 62.5%, and 95.3%, respectively, compared to the vehicle control in mice. Thus, citalopram in combination with scopolamine exhibited sub-additive effect compared to scopolamine per se and citalopram per se; similarly, fluvoxamine in combination with scopolamine exhibited sub-additive effect compared to scopolamine per se and fluvoxamine per se; however, venlafaxine with scopolamine resulted in significant (P < 0.0001) synergistic hyper-additive antidepressive-like effect compared to scopolamine per se and venlafaxine per se treatment [Figure 2].

**Effects of Various Treatments on Tail Suspension Test in Mice**

Treatment with pharmacological interventions rejected the null hypothesis in the TST and there was a difference observed within the mean values of different treatments (SS-16882, DF-8, MS-2110, F (8, 45) = 20.17, P < 0.0001). Scopolamine, citalopram, duloxetine, fluvoxamine, venlafaxine, citalopram with scopolamine, duloxetine with scopolamine, fluvoxamine

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**Table 1:**

| Dug                  | Doses in mg/kg | % decrease in immobility time | ED₅₀ in mg/kg |
|----------------------|---------------|-------------------------------|--------------|
| Citalopram           | 2             | 18.6                          | 12.5         |
|                      | 4             | 24.2                          |              |
|                      | 8             | 38.0                          |              |
|                      | 16            | 53.8                          |              |
|                      | 32            | 64.3                          |              |
|                      | 64            | 75.5                          |              |
| Duloxetine           | 2             | 13.9                          | 41.82        |
|                      | 4             | 18.8                          |              |
|                      | 8             | 27.0                          |              |
|                      | 16            | 39.4                          |              |
|                      | 32            | 54.9                          |              |
|                      | 64            | 66.4                          |              |
| Fluvoxamine          | 2             | 15.9                          | 17.5         |
|                      | 4             | 20.0                          |              |
|                      | 8             | 32.3                          |              |
|                      | 16            | 45.4                          |              |
|                      | 32            | 52.1                          |              |
|                      | 64            | 53.0                          |              |
| Venlafaxine          | 2             | 18.2                          | 15.7         |
|                      | 4             | 31.3                          |              |
|                      | 8             | 38.7                          |              |
|                      | 16            | 49.7                          |              |
|                      | 32            | 69.5                          |              |
|                      | 64            | 75.8                          |              |
Singh and Singh: Antidepressants and scopolamine

with scopolamine, and venlafaxine with scopolamine decreased the immobility time 31.1%, 47.6%, 40.9%, 42.8%, 35%, 40.5%, 60.2%, and 93.6% respectively, compared to vehicle control in mice. Only fluvoxamine in combination with scopolamine exhibited sub-additive effect compared to scopolamine per se and fluvoxamine per se whereas, venlafaxine with scopolamine resulted in significant ($P < 0.0001$) synergistic antidepressant-like effects as shown in FST compared to scopolamine per se and venlafaxine per se in mice [Figure 3].

**Effects of Various Treatments on Locomotor Activity Test**

None of the groups produced any significant change ($P < 0.050$) in locomotor activity [Figure 4] concluding the fact that the reduction in duration of immobility with test drugs was due to antidepressant effect and not because of false positive central nervous system stimulatory effect.

**Effects of Various Treatments on Step Down Test for Evaluating Memory**

In step down test, the vehicle control group showed the SDL 86.5 ± 9.7 s and none of the treatment either alone or in combination were found to significantly ($P < 0.05$) alter it. Similarly, no significant effect was observed in case of a number of mistakes made by treatment groups when compared to the vehicle control [Figure 5]. Thus, concludes that none of the treatments have any detrimental effect on memory.

**Discussion**

The combination of antidepressants are used so as to treat antidepressant-resistant depressive patients. Rogóz et al.[5] reported the synergistic interactions between various antidepressants and uncompetitive NMDA receptor antagonists in FST for the treatment of resistant depression. Molina-Hernández et al.[20] discussed various combinations of drugs like ketoconazole, fluoxetine, or desipramine along with allopregnanolone for synergistic antidepressant effect. Similarly, scopolamine, which is a muscarinic antagonist, was envisaged to show a synergistic effect with standard antidepressants in the present study. Jaffe et al.[21] have highlighted the

![Figure 2: Tail suspension test in mice. All values are represented as mean ± standard error of the mean; n = 6, *represents significant difference ($P < 0.05$) of the combination of venlafaxine and scopolamine compared with venlafaxine per se and scopolamine per se using analysis of variance Tukey’s *post-hoc* test](image)

![Figure 4: Locomotor activity in mice. None of the pharmacological intervention was responsible for increasing locomotor activity significantly compared to vehicle control. All values are represented as mean ± standard error of the mean; n = 6 and calculated using analysis of variance Tukey’s *post-hoc* test](image)

![Figure 3: Forced swim test in mice. All values are represented as mean ± standard error of the mean; n = 6, *represents significant difference ($P < 0.05$) of the combination of venlafaxine and scopolamine compared with venlafaxine per se and scopolamine per se using analysis of variance Tukey’s *post-hoc* test](image)

![Figure 5: Step down memory test in mice. None of the pharmacological intervention modulated number of mistakes significantly compared to vehicle control in mice. All values are represented as mean ± standard error of the mean; n = 6 and calculated using analysis of variance Tukey’s *post-hoc* test](image)
effectiveness of scopolamine as an antidepressant and described it as an effective agent in ameliorating both unipolar and bipolar depression, working as quickly as 3 days after initial infusion. A clinical trial study by Drevets and Furey[22] has corroborated the fact that scopolamine produces a rapid and robust antidepressant response in unipolar depressives. Therefore, scopolamine was selected in our study for the pharmacological modulation of antidepressants for ameliorating depression.

In the present study, FST and TST were used as these are the most commonly used models for depression in mice.[12,14] In addition to this step-down avoidance paradigm was performed in mice. It was very essential to evaluate the effect of drugs on retrieval of memory. This model is most commonly used to estimate the effect of drug treatments on cognition.[13] Finally, locomotor activity was performed in all the groups. As the reduction in passive behavior is interpreted as an antidepressant-like effect of the manipulation, provided it does not increase general locomotor activity, which could provide a false positive result in the FST.[18] To rule out any such possibilities in the result of the FST, locomotor activity assessment was carried out. False positive effects do occur with stimulants due to the generalized increase in activity, which are actually not antidepressants.[19]

The results of this study show that scopolamine in combination with venlafaxine but not with any other antidepressants show synergistic potential by significantly decreasing the immobility time in FST and TST. However, more research needs to be done so as to find out the path through which synergistic effect was observed only in case of venlafaxine and not in other antidepressants. Scopolamine was used at the dose of 0.2 mg/kg i.p. either alone or in combination with antidepressants which neither altered locomotor activity nor exhibited a harmful effect on memory. Thus, it concludes that 0.2 mg/kg i.p. dose of scopolamine is safe without any cognition defect in mice. Our findings were similar to previous research findings in which Ji and Zhang[21] reported that scopolamine at this dose, significantly decreased the immobility time (P < 0.001) in FST but had no influence on the locomotor activity in open field test.

The mechanism of synergism is not known and more research needs to be done, but from the literature survey, it was found that scopolamine by rapidly acting on mammalian target of rapamycin (mTOR) reverses the atrophy of spines and induces a fast onset of action with a positive role in synaptogenesis. mTORC1-i-s a well-known target for inducing rapid antidepressant.[22] Under normal conditions, calcium inside the dendrite causes the activation of mTOR signaling cascade via activation of PI3K pathway, which is responsible for basal levels of protein formation.[23] With scopolamine, GABA_A,R expression might increase leading to increase in resting L-type calcium channel activity. Thus, in turn, it may activate the mTOR signaling, leading to more mRNA translations compared to normal. The same mechanism is similarly implicated in the rapid antidepressant actions and synergism of NMDA receptor antagonists with antidepressants.[24]

Conclusions
The dose of scopolamine selected for synergistic potential has no detrimental effect on memory. No change in locomotor activity observed in any group compared to vehicle control; the results suggest the further evaluation of venlafaxine at median ED_50 over other antidepressants for combination with scopolamine in ameliorating treatment resistant depression.

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Conflicts of Interest
There are no conflicts of interest.

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