Impact on behavioral changes due to chronic use of sertraline in Wistar albino rats

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ABSTRACT

Aim: Despite having better tolerability and a wide range of clinical applications over other antidepressants, selective serotonin reuptake inhibitors (SSRIs) are also known to be associated with serious adverse effects like suicidal ideation on chronic use. The present study had explored the impact of the chronic use of sertraline, an SSRI, on the behavioral changes in Wistar albino rats.

Materials and Methods: The study was conducted on 30 Wistar albino rats of either sex; divided into five groups. Four groups were subjected to chronic mild stress induced by using various stressors randomly scheduled in a week and continued for a period of 3 weeks. The stressed rodents were subjected to sertraline treatment for 9 weeks in different human therapeutic doses extrapolated to animal doses. Behavioral changes were monitored, assessed, and evaluated throughout the treatment phase with the help of tests such as locomotor activity test, forced swim test, tail suspension test, antianxiety test, and sucrose preference test (SPT).

Results: All tests except SPT, demonstrated significant ($P < 0.05$) reduction in depressive-like activity in the stressed rodents by the mid-treatment phase, followed by an abrupt onset of the depressive state by the end of the treatment phase. SPT showed a significant ($P < 0.05$) increase in sucrose consumption throughout the treatment phase.

Conclusion: Behavioral changes following chronic sertraline administration conferred gradual remission of depression state on initial treatment phase, followed by a reversal of effect on chronic use.

KEY WORDS: Behavioral changes, chronic mild stress, selective serotonin reuptake inhibitors, sertraline

Introduction

Depression is the most common of the affective disorders ranging from a very mild condition bordering on normality, to severe depression accompanied by hallucinations and delusions. Today, depression is estimated to affect 350 million people, thus being a significant contributor to the global burden of disease. Depressive disorders often start at a young age; they affect people’s functioning; and often are recurring. For these reasons, depression is the leading cause of disability worldwide in terms of total years lost due to disability.

Antidepressants, evolved over the past 50 years, have been the mainstay of treatment of moderate to severe depression beside psychotherapy. The exact pathophysiology of depression remains largely unknown; however, most of the theories focus on the role of the neurotransmitter serotonin. The dopaminergic system has also been associated with reward and appetitive...
motivation. Serotonin has modulatory effects on dopamine by either increasing or decreasing its activity depending on the related action of other neurotransmitters and the receptor subtype it is acting on.[13]

Compounds inhibiting monoamine reuptake/breakdown, lead to an increased concentration of monoamines in synaptic clefts. Such compounds are clinically effective antidepressants,[16] selective serotonin reuptake inhibitors (SSRIs) are among the various antidepressant classes which include the tricyclic and related cyclic antidepressants, and the monoamine oxidase inhibitors.[5,6]

In January 1988, after the entry of the first SSRI, fluoxetine (Prozac) into the United States marketplace; reports began to appear describing fluoxetine-induced violence against self as well as others. In May 1990, the US Food and Drug Administration demanded the manufacturer of Prozac, Eli Lilly, and Company, to add “suicidal ideation” and “violent behaviours” to the post-introduction reports section of its label. Compared with the tricyclic antidepressants; SSRIs were then considered almost devoid of any side effects. However, questions about its safety and tolerability crept up with their continued use.[7]

Research findings reported several cases where the risk of suicidal thinking and behavior has been linked with the use of SSRI in trials and other clinical set ups.[6-13]

Preclinical research, alone cannot resolve an issue involving suicidality in humans. Though animal models cannot replicate human psychopathology in every detail, its proper conceptualization as an experimental system can help investigate selected and specific questions in ways impossible to do in humans.

Therefore, we deemed it worthwhile to investigate the behavioral changes induced by chronic SSRI treatment in an animal model. The present study was thus undertaken to monitor, assess, and evaluate the behavioral changes induced in Wistar albino rats following chronic treatment with sertraline, a leading antidepressant of SSRI class.

Materials and Methods

Animals

The study was conducted on a total of 30 Wistar albino rats of either sex weighing between 90 and 120 g. All animals have been group-housed directly on bedding in polycarbonate cages. Normal laboratory diet and water was made available ad libitum throughout the study with the exception of periods of chronic mild stress (CMS) according to the designed study protocol. They were allowed to acclimatize for 2 weeks before the study and maintained under a 12 h light and dark cycle. The experimental protocol was duly approved by the Institutional Animal Ethics Committee, and animal care 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. 1485/POL/a/11/CPCSEA).

Drugs and Chemicals

Sertraline hydrochloride (ZOSERT, Sun Pharma Pvt Ltd.), dimethyl sulfoxide (DMSO) (Qualigens Fine Chemicals) and polyethylene Glycol (PEG) (MW 400) (Thermo Fischer Scientific India Pvt Ltd.) were procured for this study. All chemicals used in this study were of analytical grade.

Grouping and Drug Treatment

Animals were divided into five groups (containing 6 animals each) as follows:
- Group 1: Normal control group
- Group 2: Stress control group
- Group 3: Treatment group (sertraline 2.58 mg/kg equivalent to human therapeutic dose of sertraline 25 mg/kg)
- Group 4: Treatment group (sertraline 5.17 mg/kg equivalent to human therapeutic dose of sertraline 50 mg/kg)
- Group 5: Treatment group (sertraline 10.33 mg/kg equivalent to human therapeutic dose of sertraline 100 mg/kg).

The vehicle = 50% DMSO, 25% PEG (molecular weight 400), and 25% distilled water.

Experimental Procedure

Initially, the animals were given 1% sucrose solution in their home cages with no food or water for a period of 48 h, which was followed by an 18 h session of food and water deprivation. Then, they were given sucrose for 1 h/day on 5 consecutive days. The total sucrose intake was measured at the end of the training period in order to group the rodents. The animals in the experimental groups were then subjected to CMS for a period of 3 weeks.

Chronic Mild Stress Induction

The CMS procedure consisted of a range of unpredictable mild stressors randomly scheduled over a 1 week period and repeated throughout the 3 weeks experiment (Table 1).[14] The stressors included tilted cage (45°), restricted food access, wet cage, empty bottle exposure, crowded housing, food and water deprivation, and novel odor exposure. Thus, stressors were presented both during the rats’ active (dark) and inactive (light) period.

After 7 days of stress induction, animals were treated with the test compound and/or vehicle intraperitoneally. Behavioral parameters were measured after 9 weeks of treatment. The observer was blinded to all the treatments to remove any possible observer bias. Each animal was subjected to following tests:

Locomotor Activity Test

Rodents were placed in a digital photoactometer. A continuous beam of light (from about 6 lights) were made to pass through the animal cages. The animals were observed for a period of 60 minutes and total activity of the animals was expressed in terms of number of entries and movements. Measurements were done for 3 days.

Table 1:

Schedule for CMS induction in Wistar albino rats

| Day | Inactive (light) phase | Active (dark) phase |
|-----|-----------------------|---------------------|
| 1   | C                     | A                   |
| 2   | E                     | B                   |
| 3   | F                     | C                   |
| 4   | G                     | D                   |
| 5   | E                     | F                   |
| 6   | G                     | E                   |

A=Tilted cage (45°) (3 h), B=Restricted access to food (1 h), C=Wet cage (200 ml water in 100 g bedding) (21 h), D=Exposure to empty bottle (1 h), E=Crowded housing (10 animals per cage) (18 h), F=Food and water deprivation (18 h), G=Exposure to novel odor (household air freshener (3 h), CMS=Chronic mild stress
fall on the photoelectric cells, which gets activated when the rodent crossed the beam of light and thereby cutting it off. The total cut-offs were counted for a session of 10 min, and the figures served as an index of locomotor activity of the animal.[15]

**Forced Swim Test**

Pretreated rodents were individually forced to swim in an open cylindrical vessel. The total amount of time each rat remained immobile during a 5 min session of forced swim was recorded as the immobility time. The rats were adjudged to be immobile when they ceased struggling and remained motionlessly floating in the water. Each rat underwent a pre-swim session 24 h prior testing.[16]

**Tail Suspension Test**

Pretreated rats were individually suspended from the edge of a shelf 58 cm above a table top by an adhesive tape fixed approximately 1 cm from the tip of the rodent’s tail. The period of immobility was recorded during a session of 5 min. A rodent was considered immobile if it hangs passively and completely motionlessly for at least 1 min.[17]

**Antianxiety Test**

The testing apparatus consisted of a light and a dark chamber divided by a photocell-equipped zone. A polypropylene animal cage, 44 cm × 21 cm × 21 cm, was darkened with black spray paint over one-third of its surface with a partition (13 cm × 5 cm) separating the dark one-third from the brightly illuminated two-third portion of the cage. An electronic system consisting of 4 sets of photocells across the partition automatically counted the movements through the partition and clocked the time spent in the light and dark chambers respectively. Pretreated 30 min before the experiment, animals were placed into the cage and observed for a 10 min period. The numbers of crossings through the partition were compared with total activity counts during the period.[18]

**Sucrose Preference Test**

Rats were given free access of their standard laboratory rodent diet. On the 1st day of the test or the day of habituation; each cage were supplied with two identical graduated water bottles containing 250 ml of water in each. On the following day of the test, regular water in one of the bottles was replaced with sucrose solution (0.1% sucrose diluted in regular water). The test was conducted for 24 h. Taste preferences were expressed as the percent of the volume of sucrose solution of the total volume of sucrose solution consumed within a 24 h period.[19]

**Statistical Analysis**

Data have been expressed in mean ± standard error of mean (SEM). Differences among groups were compared using one-way ANOVA through statistical software such as Statistical Package for the Social Sciences (SPSS) [SPSS Statistics for Windows Version 17.0. Chicago: SPSS Inc] and Microsoft Excel. Tukey’s range test was used as a post-hoc test for comparison among groups. Statistical significance was set at $P < 0.05$ with a 95% confidence interval.

**Results**

**Locomotor Activity**

The mean ± SEM locomotor activity count of the stress control group of rats were found to be significantly ($P < 0.01$) lower when compared with the normal control rats. Post-hoc Tukey’s Range tests revealed that the changes in the locomotor activity count of the stress rats of the treatment groups were significant ($P < 0.05$) in comparison to the stress control group and the normal control group, respectively [Figure 1].

**Forced Swim Test**

The mean (±SEM) immobility time of the stress control group of rats were found to be significantly ($P < 0.01$) higher when compared to the normal rats. Post-hoc Tukey’s Range tests revealed that the changes in the time of immobility of the stress rats of the treatment groups were significant ($P < 0.05$) in comparison to the stress control group and the normal control group, respectively [Figure 2].

**Tail Suspension Test**

The mean (±SEM) immobility time of the stress control group of rats were found to be significantly ($P < 0.01$) higher when compared to the normal rats. Post-hoc Tukey’s range tests revealed that the changes in the time of immobility of the stress rats of the treatment groups were significant ($P < 0.05$) in comparison to the stress control group and the normal control group, respectively [Figure 3].

**Antianxiety Test (Dark Light Model)**

**Time spent in dark chamber**

The mean (±SEM) time spent in the dark chamber by the stress control group of rats were found to be significantly ($P < 0.01$) higher when compared with the normal rats. Post-hoc Tukey’s range tests revealed that the changes in the time spent in the dark chamber by the treatment groups were significant ($P < 0.05$) in comparison to the stress control group and the normal control group, respectively [Figure 4].

**Time spent in light chamber**

The mean (±SEM) time spent in the light chamber by the stress control group of rats was found to be significantly ($P < 0.01$) lower when compared with the normal rats. Post-hoc Tukey’s range tests revealed that the changes in the time spent in the light chamber by the stress rats of the treatment groups were significant ($P < 0.05$) in comparison to the stress control group and the normal control group, respectively [Figure 5].

**Sucrose preference test**

The mean (±SEM) sucrose consumption of the stress control group of rats were found to be significantly ($P < 0.01$) lower when compared to the normal rats. Post-hoc Tukey’s range test for comparison among groups was found to be significantly ($P < 0.05$).
In the present study, forced swim activity, and periodic thoughts of death or suicidal ideation. Most antidepressant medications increase the levels of monoamines in the synaptic cleft between neurons in the brain. Evidence from continued research supports the association of the serotonergic system in the pathophysiology of depression. Despite having better tolerability and a wide range of clinical applications over other antidepressants, SSRIs are also known to be associated with serious adverse effects like suicidal ideation on chronic use. This urged us to attempt an animal study to probe behavioral changes in a rodent model following prolonged treatment with sertraline at different human therapeutic doses extrapolated to animal doses. Behavioral changes observed through various screening tests such as locomotor activity test; forced swim test (FST), tail suspension test (TST), antianxiety test and sucrose preference test (SPT) served as an index for estimating the depressive profile.

Locomotor activity is an index of alertness and muscle relaxation. Decrease in motor activity is an indication of central nervous system depressant property. In the present study, the locomotor activity of the stress control rats was found to be decreased in comparison to the normal control rats. On treatment with sertraline at different doses, it was found that the mean locomotor activity of Group 3 gradually increased until till week 4, following a steep fall by week 5.

Counts for Group 4 and 5 were found to be in an increasing trend till week 6, followed by a sharp fall by week 7, while that activity in Group 1 and 2 was found to be decreased in comparison to the normal control rats. On treatment with sertraline at different doses, it was found that the mean locomotor activity of Group 3 gradually increased until till week 4, following a steep fall by week 5.

The FST and TST models of depression are widely used to screen any novel antidepressant drugs due to their relative sensitivity and specificity to all major classes of antidepressant drugs, including tricyclics, serotonin specific reuptake inhibitors, monoamine oxidase inhibitors, and atypicals. In our study, when recording the time of immobility of different groups of rats for forced swim activity, it was observed that there was an increase in mean time of immobility of stress control rats when compared to the normal control rats. On treatment with sertraline at different doses, it was found that the mean immobility time gradually decreased in Group 3 till week 6. This was found to be in accordance with a study by West et al., where forced swim activity was observed after administration of paroxetine,

**Discussion**

Depression, a heterogeneous disorder, is a major cause of morbidity worldwide with people experiencing a lack of interest and pleasure in daily activities, significant weight loss or gain, insomnia or hypersomnia, fatigue, inability to concentrate, and periodic thoughts of death or suicidal ideation. Most antidepressant medications increase the levels of monoamines in the synaptic cleft between neurons in the brain. Evidence from continued research supports the association of the serotonergic system in the pathophysiology of depression.
Figure 6: Different doses of sertraline showing effect on sucrose consumption in rats subjected to sucrose preference test (one-way ANOVA followed by post-hoc Tukey’s range tests; $P < 0.05$)

The underlying mechanism behind this behavior can be faintly postulated. SSRIs acts by inhibiting the reuptake of serotonin which then remains in the synaptic gap longer than usual, thereby possibly stimulating the receptors of the recipient cell frequently. In the short-term, this leads to increase in signaling across a synapse in which serotonin serves as the major neurotransmitter. On chronic treatment, the sensitivity of these 5-hydroxytryptamine (5-HT) neurons to 5-HT$_{1A}$ receptor agonist decreases gradually thus causing a progressive desensitization of 5-HT$_{1A}$ autoreceptors. After a decrease in the initial weeks, the electrical activity of 5-HT neurons of dorsal raphe nuclei possibly reverts to its initial levels after chronic treatment. This therapeutic delay in the efficacy is reported to be due to the time necessary for a persistent deactivation of 5-HT$_{1A}$ autoreceptors.$^{24}$ However, mechanisms behind reversion of depressive state from the mid-treatment phases still remains unclear.

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Conflicts of Interest
There are no conflicts of interest.

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