Prenatal Olanzapine Induced Behavioural Changes in Swiss Albino Mice

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ABSTRACT

BACKGROUND

Olanzapine is an atypical antipsychotic agent and is one of the most commonly used drugs for episodes of psychosis. Women with psychosis and on medications for the condition may become pregnant. The safety profile of olanzapine has not yet been completely established during pregnancy. We wanted to study the behavioural changes in pups of swiss albino mice who received Olanzapine during gestational period.

METHODS

The study was performed in the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Thirty adult female Swiss albino mice of weight 20 - 25 gms and average age of 80 - 100 days, were utilized after approval of institutional ethical committee. The temperature of animal room was kept ambient and relative humidity of 50 - 60% was maintained with 12 hr of light and 12 hr of dark cycle. In the evening the female mice who were in their pre-estrous phase were transferred to the cages containing male mice in ratio of 2:1. In the morning the pregnancy was checked for by the presence of vaginal plug and was designated as day zero of gestation. All pregnant mice were divided into three equal groups of 10 mice each. Group 1 was treated with Olanzapine at dose 2 mg/Kg orally throughout gestation period. Group 2 was treated with Olanzapine at dose 6 mg/Kg orally throughout gestation period. Group 3 was given equal amount of tap water at the same time via the same route. Animals from all the three groups were allowed to deliver. The delivered pups of the three groups were subjected to behavioural tests namely open field test, elevated plus maze test, and Morris water maze test, at the age of 8 weeks approximately to measure exploratory, anxiety, memory and learning behaviour.

RESULTS

Olanzapine treated mice for the dose 2 mg/Kg showed heightened activity and reduced anxiety in open field and elevated plus maze test as compared to control. However, for dose 6 mg/Kg the offspring showed heightened anxiety and fearfulness as compared to controls. The low dose group had improved memory and learning behaviour whereas in high dose it was distorted.

CONCLUSIONS

Above findings suggest that Olanzapine has a deleterious effect on nervous system development when given in high doses, thus resulting in abnormal anxiety states, possibly due to its potent activity at dopamine, serotonin, muscarinic, histamine and adrenergic receptors.

KEYWORDS

Antipsychotics, Exploratory Behaviour, Dopamine, Schizophrenia, Histamine Receptors

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BACKGROUND

Women having psychiatric conditions may become pregnant, and motherhood is common in such women. Women with psychotic disorders who are pregnant or breastfeeding are often treated with antipsychotics.

Olanzapine is a second-generation antipsychotic primarily used for Schizophrenia and bipolar disorders. It acts on dopamine and serotonin receptors. It is a thieno-benzodiazepine analog with the chemical name of 2-methyl-4-(4-methyl-1-piperazinyl)-10-thieno[2,3-b][1,5]-benzodiazepine. It blocks dopamine from having an exaggerated action thus reducing the positive symptoms of patients of schizophrenia like delusions, hallucinations and disrupted speech, thought and behaviour. It also has an antagonistic action on serotonin 5HT2A receptors which reduces the negative symptoms of schizophrenia namely, poor attention, flat effect, alogia, avolition anhedonia.[1] Olanzapine has potent antagonism for 5-$HT_{2A/2C}$, 5-HT_3, and 5-HT_6 receptors. It also bears antagonism for dopamine D_1, D_2, D_3 and D_4 receptors and selective muscarinic-binding sites.[2] Its antagonism to muscarinic receptors may explain its anticholinergic properties

Olanzapine has higher affinity to 5-HT_{2A} receptors than D_2 receptors (high 5-HT_{2A}/D_2 ratio). In comparison to the other atypical antipsychotics, olanzapine presents highest affinity for histamine H_1 receptors, high affinity for serotoninergic 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, and 5-HT_6 receptors, medium affinity for dopaminergic D_1, D_2, D_3, D_4, D_5, and muscarinic M_1–M_5 receptors, low affinity for adrenergic α_1 and α_2 receptors.[3]

The animal behavioural and electrophysiological studies show that at low doses, olanzapine might act as an atypical antipsychotic whereas at very high doses, it might resemble the typical antipsychotic.

While there is limited evidence quantifying the extent to which these drugs are transferred across the placenta, a study suggests in the range of 5–14% of a labeled olanzapine can be transferred from maternal to fetal system in 4h.[4] However, the effects of atypical antipsychotics on fetal growth may be also mediated via altered placental development and/or function.[5] There are very few and non-conclusive reports about the teratogenicity of olanzapine. The present study has aimed to see the behavioural effects of swiss albino mice when olanzapine was given at different doses prenatally. The exploratory behaviour of animal was assessed by open field test[6] Its anxiety level was assessed by elevated plus maze test,[7] The memory and learning behaviour was assessed by Morris water Maze test.[8] It has been suggested by literature that on gestational days 15- 16 there is peak of cell differentiation for serotoninergic neurons and by day 19 the distribution of serotonin neurons resembles that found in the adult.[9] Thus olanzapine which act on serotonin receptors might hamper the development of serotonergic system in this critical period.

This study aims to find out the effect of Olanzapine on the behaviour of pups of Mice who received the drug during gestational period.

METHODS

The study was performed in the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Thirty adult-female swiss albino mice of weight 20–25 gms and average age of 80–100 days, were taken after approval of the Institutional Ethics Committee and Central Animal Ethics Committee. The temperature of animal room was kept ambient and relative humidity of 50-60% was maintained with 12 hrs. of light and 12 hrs. of dark cycle.

Cages made of polypropylene material and having rice husk as bedding were chosen to house the mice. They were kept on a pelleted diet which was obtained from local Pashu Ahar Kendra and tap water ad libitum. In the evening the female mice who were in their pre estrous phase were transferred to the cages containing male mice in ratio of 2:1. In the morning the pregnancy was checked by the presence of vaginal plug and was designated as day zero of gestation. All pregnant mice were divided into following groups-

Group 1
Treated with Olanzapine at dose 2 mg/Kg orally throughout gestation period from day 0 till birth.

Group 2
Treated with Olanzapine at dose 6 mg/Kg orally throughout gestation period from day 0 till birth.

Group 3
Not treated with Olanzapine throughout gestation period from day 0 till birth.

Animals from all the three groups were allowed to deliver. These delivered pups were subjected to various behavioural tests at the age of 8 weeks approximately. We wanted to test following parameters in all the three groups of mice -

1. Exploratory behaviour of mice.
2. Anxiety levels of mice.
3. Memory and learning behaviour of the mice.

To test the exploratory behaviour of mice open field test (Figure 1A) apparatus was used. Only the apparatus was illuminated and rest of the room was kept dark. Pups of each group were individually kept in the apparatus for a duration of 5 mins and during that time following activities were noted.

1. The number of squares crossed by the mice i.e. Ambulation.
2. The number of times mice stood on its hind limbs i.e. rearing.
3. The number of times the mice did action of licking, grooming, scratching i.e. self-grooming.
4. Duration for which mice remains still without any movement i.e. freezing time.
5. The number of faecal pellets excreted by each individual mice during the trial of 5 minutes.

Figure 1. A - Open Field Test - The experimental mice (↓) seen in one of the 16 quadrangles, B - Elevated Plus Maze Test - The experimental mice (←) placed on the open arm of elevated plus maze. On the sides are the closed arms (*), C - Morris Water Test - The mice are placed on elevated platform (←) poles 3 (yellow mark) and pole 4 (red mark) are visible. Pole 1 is facing opposite to pole 3 and pole 2 is facing opposite pole to pole 4

To test the anxiety levels of mice of each group elevated plus maze test (Figure 1B) was performed. The experimental animals were placed individually in the center of maze facing an enclosed arm and the time spent on the open and closed arms were recorded during the next 5 min for each mice. An arm entry was recorded when all four paws of the rat entered the arm. To test the memory and learning behaviour of the mice Morris water maze (Figure 1C) test was used. It consist of a black circular pool with water maintained at 25 ^{0}C. Few drops of India ink is added to the water to turn it opaque. On first day the mice was exposed to the apparatus and water in it for 1 minute. On next day the maze was divided into 4 quadrants by marking 4 poles on the perimeter of the pool. A rectangular platform of 9 cm diameter was kept hidden below in the center of one of the quadrants. Each mice pup was placed in the water at one of the poles which was considered as the starting location and was allowed 90 seconds to find the hidden platform where it was allowed to rest for 20 seconds. After that it was lifted out and again the same sequence of testing procedure was started from second next pole. This was repeated for third and fourth poles also. The time taken by the animal to find the hidden platform was noted in all the four trials. These tests were repeated in three sessions each one separated from each other by duration of 4-5 hours.

All the data recorded were entered into excel sheet and was statistically analyzed by applying one-way ANOVA followed by post hoc Tukey test using software SPSS (statistical package for social sciences) trial version 21.

RESULTS

Open Field Test

There was significant increase in ambulation, rearing, self-grooming, number of faecal pellets of Group 1 (low dose) as compared to Group 2 (high dose). There was increased immobility period in Group 2 compared to Group 1 (Table 1).

Elevated Plus Maze Test

During this test offspring of group 1 mice were found to spend significantly more time in open arms as compared to offspring of group 3 (control) and group 2. Of all the groups offspring of group 3 spent least time in open arms. In addition, group 2 mice, were observed to spend significantly more time in enclosed arms as compared to group 1 and group 3. On comparing the number of entries in the open arms and closed arms by the offspring of mice of all the 3 groups, offspring of group 1 showed significantly more

	Group 3 vs 1 P Value	Group 3 vs 2 P Value	Group 1 vs 2 P Value
Ambulation	0.066	0.708	0.013*
Rearing	<0.001***	0.650	<0.001***
Self-Grooming	0.001**	0.923	0.001**
Immobility Period	0.171	0.728	0.045*
Fecal Palates	0.145	0.396	0.011*

Table 1. Group Comparison of Open Field Exploratory Behaviour in Mice Offspring

* The difference in the groups will be considered statistically significant only when p<0.05.
** The difference in the groups will be considered statistically significant only when p<0.01.
*** The difference in the groups will be considered statistically significant only when p<0.001.

	Group 3 vs 1 P Value	Group 3 vs 2 P Value	Group 1 vs 2 P Value
Time spent in Close arm	0.020*	0.029*	<0.001***
Time spent in Open arm	0.026*	0.008**	<0.001***
Time taken to enter close arm	0.011*	0.874	0.004**
Time taken to Enter open arm	<0.001***	0.272	<0.001***

Table 2. Group Comparison of Elevated Plus Maze Test Behaviour in Mice Offspring

* The difference in the groups will be considered statistically significant only when p<0.05;
** The difference in the groups will be considered statistically significant only when p<0.01;
*** The difference in the groups will be considered statistically significant only when p<0.001
Morris water maze test:

	Group 3 vs Group 1	Group 3 vs group 2	Group 1 vs Group 2
Session 1	0.588	0.864	0.313
Session 2	0.061	0.266	0.002**
Session 3	0.275	0.513	0.040*

Table 3. Group Comparison of Morris Water Maze Test Behaviour in Mice Offspring

*The difference in the groups will be considered statistically significant only when p<0.05.
**The difference in the groups will be considered statistically significant only when p<0.01.

entries in the open arms as compared to group 2 and group 3. But their entries in closed arms were greatly reduced in comparison to group 2 and 3. (Table 2)

Morris Water Maze Test

This experiment was done in three sessions of escape latencies, followed by one session of probe trial and new platform trial. In session 1 of our experiment no significant difference was found in the time taken by offspring of different groups i.e. group 1, 2 and 3 to find hidden platform. They took approximately same time to reach hidden platform. In session 2, the offspring of mice of different groups showed significant difference in the time to reach platform. In this session the offspring of the mice of group 1 took less time to reach platform than the offspring of group 2 and 3. The group 2 mice offspring took maximum time to reach the hidden platform. In session 3 also the different mice groups followed same pattern of time duration, but the time taken by all of them to reach platform was reduced further than in session 2.

DISCUSSION

In mammalian embryos, development starts with rapid multiplication of cells which show little, if any, differentiation and this period extends from fertilization to germ layer formation. This pre-differentiation stage is followed by embryonic period when cells show distinct morphological differences also at chemical level and then comes the final fetal period which is characterized by rapid organ differentiation and growth. Every organ passes through its most susceptible stage early in differentiation in due succession [10]

Thus, development of mouse is divided into 3 Stages

1. Preorganogenesis period–gestational day 1 to 6.
2. Embryonic period–gestational day 6 to 15.
3. Fetal period–gestational day 15 to 19.

On behavioural examination there appears to be reduced anxiety in pups treated with low dose of olanzapine. The findings are comparable with those of Onaolapo OJ et al (2017)[11] and Wasik et al (2019).[12] The olanzapine had anxiolytic effect on the mice. On the other hand, heightened anxiety and deficient locomotive behaviour on high dose administration of Olanzapine may be due to heightened affinity for D_2 receptors in mesolimbic and nigrostriatal pathways leading to impaired dopaminergic activity at the same level.[13] Also the oxidative stress in mesolimbic region might lead to decreased density of pyramidal cell leading to behavioural anomalies at high dose. The findings of improved exploratory and learning behaviour in pups treated with low dose of Olanzapine is comparable to findings of that of Jae Chun Song et al (2016).[14] They reported olanzapine has a protective effect against cognitive impairments.

In Morris Water Maze experiment, high dose Olanzapine treated mice shows defective spatial learning and memory as compared to the control mice. Spatial learning and memory is controlled by the circuitory between hippocampus and orbitofrontal cortex involving Papez circuit via fornix. Again the reactive oxygen species induced neuronal damage by creation of MDA and superoxide which are cytotoxic, may be the possible mechanism inducing this effect.[15] A systematic review carried out by Ennis ZN, Damkier P. reported that first-trimester exposure to olanzapine is not associated with an increased risk of congenital malformation.[16]

CONCLUSIONS

Olanzapine is a commonly used atypical antipsychotic for schizophrenia and bipolar disorder. The clinician should discuss with the patient who is pregnant or desires to become pregnant regarding all the risks associated with the drug. It should be prescribed only when benefits outweigh risks for both the mother and foetus.

Limitations

The study was done on Swiss albino mice. Human studies are yet to be done.

Financial or Other Competing Interests: None.

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