EFFECT OF OLANZAPINE ON A MODEL OF SCHIZOPHRENIA INDUCED BY ISOLATION REARING IN MALE SPRAGUE DAWLEY RATS

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Abstract

Schizophrenia is a common clinical disorder of psychopathology. Olanzapine has proven efficacy to treat positive symptoms of schizophrenia with enhanced tolerability compared to older first generation antipsychotics. The present study was undertaken to explore the possible effects of olanzapine (6 mg/kg/day) on sociality and cognition in isolated reared rats as a model of schizophrenia. **Results:** It could be seen that sociality (SI)is deteriorated significantly in isolated reared rats which is very suggestive of induction of schizophrenia's negative symptoms and significantly improved by chronic administration of olanzapine to the extent of social reared (SR) group. Chronic administration of olanzapine obviously decreased cognition indicated by decreased (SPI), increased latency to reach target quadrant and decreased % of time spent in target quadrant day **Conclusion**: olanzapine had a beneficial effect on increasing sociality in isolated reared rats, but it deteriorates spatial memory and novel object recognition.

SPI: social preference indices.

SI: Sociability index

1. Introduction

Schizophrenia is a clinical disorder of psychopathology that involves emotion, perception, and other aspects of behavior. The main symptom of schizophrenia is psychosis (positive symptom), other manifestations include negative symptoms and

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cognitive dysfunction[1], The lifetime prevalence of schizophrenia has generally been estimated to be approximately 1% worldwide[2].

Rat of isolation rearing (IR) is a commonly used method in which rat pups from the age of weaning were placed in separate cages from littermates. Generally, they are allowed to see, smell, and hear other rats but not to have social contact with them. The IR rats exhibit profound psychological, behavioral, and neurochemical changes when they grow up similar to the full-blown symptoms of schizophrenia in patients' adulthood. IR rats are thus suitable for modeling mental disorders with a pathoetiology based on developmental hypothesis[3].

Second generation antipsychotic drugs (SGAs) currently form the first line of treatment for schizophrenia[4], Among them, olanzapine has proven efficacy to treat schizophrenia with enhanced tolerability compared to older first generation antipsychotics[5]. Its most serious side effects, however, are substantial weight gain, increased adiposity and other metabolic disorders [6].

The present study was undertaken to explore the possible effects of olanzapine (6 mg/kg/day) on behavioral activities measured in a model of schizophrenia induced by isolation rearing in male Sprague Dawley rats.

2. Materials and methods

2.1. Experimental animals: All animal procedures were approved by the Institutional Animal Ethics Committee for Ain Shams University, Faculty of Medicine. Male Sprague-Dawley rats (weighing 35-40 g) were purchased from National Research Institute (Cairo, Egypt).They were housed in an animal room with temperature (22 °C) and lighting (12 h light–dark cycle) control. An adaptation period of 1 week was allowed before initiation of the experimental protocol. Upon arrival, all animals were assigned randomly to one of two housing conditions for the entire study: isolation rearing or social rearing. Isolation-reared animals were housed singly (to induce schizophrenia); socially reared animals were housed in groups of 3 per cage (control group). The duration of the experiment was 8 weeks.

2.2. Drugs &chemicals: Olanzapine (6 mg/kg/day) was purchased from Multi-Apex pharma[7], Egypt in the form of yellow powder. Rat chow (was purchased from Meladco for Animal Food, Egypt) in the form of pellets.

2.3. Experimental procedures

2.3.1. Induction of Schizophrenia by Social Isolation (Isolation Rearing): The setting for social isolation, was such that each animal of the isolation-reared rats was housed in a single transparent plastic cage. As both isolated and socially reared rats were kept in the same room, isolated rats were able to hear, smell and see other rats. All rats had free access to food[8].

2.3.2. Study design: Rats were divided into 3 groups, 6 animals each.

-Social reared group: rats fed standard chow diet for 8 weeks.

-Isolated reared group: isolated for 8weeks.

-Isolated reared olanzapine treated group: isolated for 8 weeks; received olanzapine 6mg/kg/day i.p. in the last 3 weeks **.**

2.3. Parameters measured

At the end of the experiment these parameters were measured

1- **Behavioral parameters**:

a- **Three-Chamber Social** interaction Test consists of three parts[9], conducted in three-chamber (three-compartment) apparatus

1-Habituation (adaptation), the subject was habituated for 5 minutes.

2-After habituation Social Affiliation Aspect of the Test (session I), a stranger animal (same age with no previous contact with the subject) of the same strain was introduced inside the wire cage of the left or right compartment (stranger zone 1) randomly, while the other wire cage was empty (empty zone) for the 10 min sociability test. Time spent in the stranger zone 1 and around the cage was measured versus time spent in the empty zone.

3-Social Novelty/preference Session of the Test (Session II)was conducted for another 10 minutes directly after the termination of the sociability test. Another stranger animal was introduced in the wire cage of the opposite compartment (stranger zone 2) and same parameters were measured as with the previous session to find a preference of the subject animals to the novel over the familiar animal in the wire cage. Sociability index (SI) and social preference indices (SPI) were calculated using the following formulas

$$SI = \frac{time\ spent\ in\ stranger\ zone\ 1}{time\ spent\ in\ empty\ zone}$$

$$SPI = \frac{time\ spent\ in\ stranger\ zone\ 2}{time\ spent\ in\ stranger\ zone\ 1}$$

b- **Morris Water Maze**

Morris water maze[10], is a well-validated and highly sensitive test of rodent cognitive function. Testing was conducted in the Morris water maze (diameter 1.8 m) with an ‘‘Atlantis platform’’ (diameter 10 cm).The platform was located in the center of the northeast quadrant of the pool throughout testing. The testing room contained a number of constant, salient visual cues (posters, objects, and equipment). A video camera was mounted on the ceiling directly above the pool to record the swim path of each rat.In spatial training procedure[11],rats received four trials in the Morris water-maze on each of 5 days, without any cues signaling platform location. During testing, the submerged platform remained stationary in one quadrant of the maze, and the latency to find it was determined. In this and all ensuing experiments, each trial consisted of an individual rat being placed carefully into the water, facing the outer edge of the pool, at one of four possible starting points (e.g., north, south, east, and west). The starting location for each trial was determined randomly, with the provision that all start locations were used in a given day. A trial was terminated and the latency was recorded when the rat reached the platform and remained on it for 10 sec. If the rat did not reach the platform within 120 sec, the trial was terminated, and the rat was placed on the platform for 10 sec. Thereafter, rats were transferred to a dry holding cage where they remained for 60 sec

until the next trial. After training, rats were returned to their home cages. On the sixth day, rats received an additional 60 sec probe trial in which a platform was not present within the pool. Rats were placed in the pool, as before, and latency to reach the target quadrant and the time spent within the quadrant of the platform were recorded.

Statistical analysis:was carried out using Graphpad prism, software program, version 5.0. (2007). Inc., CA, USA. All values in the results were expressed as means ± SD. Statistical difference among groups were determined using one way analysis of variance (ANOVA) followed by Dunn's multiple comparison test. But for latency to reach target quadrant two way ANOVA test was used with Bonferroni's post-hoc testP values < 0.05 were considered statistically significant.

3. Results

3.1. THREE CHAMPER SOCIAL INTERACTION TEST

a) Session 1 (Sociability Index Measures)

Table (1) demonstrate the reversal of isolation rearing-induced decrease in sociability index (SI) after three weeks of intraperitoneal (i.p.) administration of 6 mg/kg/day olanzapine to male Sprague-Dawley rats continuously exposed to the isolation rearing.It could be seen that isolation rearing decreased the SI compared to the social reared (SR) group.

On the other hand, administration of olanzapine 6 mg/kg, reversed this decrease by compared to the isolated reared (IR) group.

The differences among these groups were statistically significant ($p < 0.05$) as calculated by Kruskal-Wallis ANOVA test. Applying the Dunn's multiple comparisons test shows that isolated reared group was significantly ($p < 0.05$) different from social reared, whereas olanzapine (OLA) group was significantly ($p < 0.05$) different from isolated reared (IR) group and insignificantly ($p > 0.05$) different from social reared (SR) group.

	SI		
	SR (*n* = 6)	**IR (*n* = 6)**	**OLA (*n* = 6)**
Mean±SD	**1.95±** 0.68	**0.12±** 0.12*	**2.43±** 1.09★
ANOVA	p < 0.0002		

✳**,** significant difference (Dunn's test), at the level of 0.01 vs. social group.

★**,** significant difference (Dunn's test), at the level of 0.01 vs. isolated reared group.

n, number of animals.

SI, Sociability index.

ANOVA, analysis of variance.

b) Session 2 (Social Preference Indices)

Table (2) demonstrate the effect after three weeks of intraperitoneal (i.p.). administration of 6 mg/kg/day olanzapine to male Sprague-Dawley rats continuously exposed to the isolation rearing on cognition indicated by social preference indices (SPI).

It could be seen that administration of olanzapine 6 mg/kg decreased the (SPI) compared to the isolated reared group.

The differences among these groups were statistically significant ($p < 0.05$) as calculated by Kruskal-Wallis ANOVA test. Applying the Dunn's multiple comparisons test, shows that olanzapine group was significantly ($p < 0.05$) different from isolated reared (IR) group.

The isolated reared (IR) group was insignificantly ($p > 0.05$) different from the social reared (SR) group.

	SPI		
	SR (*n* = 6)	**IR (*n* = 6)**	**OLA (*n* = 6)**
Mean±SD	**2.69**± 1.89	**3.26**± 1.28	**0.09**± 0.036★
ANOVA	p < 0.0002		

★, significant difference (Dunn's test), at the level of 0.05 vs. isolated reared group.

n, number of animals.

SPI, social preference indices.

ANOVA, analysis of variance.

3.2. Morris Water Maze

a) Latency To Reach Target Quadrant Measured By (sec)

Table (3)demonstrate the effect after three weeks of intraperitoneal (i.p.) administration of 6 mg/kg/day olanzapine to male Sprague-Dawley rats continuously exposed to the isolation rearing on spatial learning indicated by latency to reach target quadrant (sec).

It could be seen that administration of olanzapine 6 mg/kg increased the latency to reach target quadrant (sec) in days 1, 2, 3, 4, 5 and 6 compared to the isolated reared.

The differences among these groups were statistically significant ($p < 0.05$) as calculated by Two way ANOVA repeated measures test. Applying the Bonferroni's

multiple comparisons test, shows that olanzapine (OLA) group was significantly ($p < 0.05$) different from isolated reared (IR) group.
The isolated (IR) reared group was insignificantly ($p > 0.05$) different from the social reared (SR) group.

	Latency To Reach Target Quadrant (sec)		
	SR (*n* = 6)	**IR (*n* = 6)**	**OLA (*n* = 6)**
Mean±SD **Day1**	**72.80**± 22.11	**86.25**± 23.56	**120.00**± .00★
Mean±SD **Day2**	**49.20**± 28.29	**49.00**± 31.14	**120.00**± .00★
Mean±SD **Day3**	**33.20**± 18.19	**38.25**± 26.87	**120.00**± .00★
Mean±SD **Day4**	**20.40**± 7.96	**31.75**± 32.42	**120.00**± .00★
Mean±SD **Day5**	**13.00**± 7.25	**20.75**± 24.90	**120.00**± .00★
Mean±SD **Day6**	**11.00**± 9.80	**25.50**± 27.67	**60.00**± .00★
ANOVA	$p < 0.0001$		

★, significant difference (Bonferroni's test), at the level of 0.001 vs. isolated reared group

n, number of animals.

ANOVA, analysis of variance.

b) % Of Time Spent In Target Quadrant Day 6

Table (4)demonstrate the effect after three weeks of intraperitoneal (i.p.) administration of 6 mg/kg/day olanzapine to male Sprague-Dawley rats continuously exposed to the isolation rearing on memory indicated by % of time spent in target quadrant.
It could be seen that administration of olanzapine 6 mg/kg , decreased % of time spent in target quadrant in day 6 compared to the isolated reared (IR) group.
The differences among these groups were statistically significant ($p < 0.05$) as calculated by Kruskal-Wallis ANOVA test. Applying the Dunn's multiple comparisons test, shows that olanzapine (OLA) group was significantly ($p < 0.05$) different from isolated reared (IR) group. The isolated (IR) reared group was insignificantly ($p > 0.05$) different from the social reared (SR) group.

	% Of Time Spent In Target Quadrant Day 6		
	Social reared (*n* = 6)	**Isolated reared (*n* = 6)**	**Olanzapine (*n* =6)**
Mean±SD	**19.67**± 9.88	**15.00**± 8.819	**0.0**± 0.0★
ANOVA			*p*< 0.0001

★significant difference (Dunn's test), at the level of 0.05 vs. isolated reared group.

n, number of animals.

ANOVA, analysis of variance.

4. Discussion

The present work was conducted to investigate the potential beneficial effects of olanzapine (6mg/kg/day) for 3 weeks on sociality and cognition in a model of schizophrenia induced by isolation rearing.The results of the present study showed that exposure to isolation rearing induced schizophrenia symptoms manifested by significant decline in sociality index (SI) in social interaction test (Session I) Similar results obtained in previous studies[12]. Moreover, our study proved that there is no cognitive deficits in spatial learning and memory revealed by Morris water maze (MWM) in rats that are exposed to isolated rearing as well as social rearing. This is in agreement with previous studies that reported improved spatial learning and memory in visuospatial learning task in isolated reared rats [13], Also isolated reared rats performed as well as social reared, exploring the novel object significantly more than the familiar object in Social Novelty/preference Session of social interaction test (Session II)as no impairment in novel object discrimination occurs in isolation-reared rats using short inter-trial intervals such as 1–15 min[14].

The present study showed that olanzapine treatment for 3 weeks reversed the schizophrenia like symptoms by significant increase in SI in three chamber social interaction test The increased sociality effect of olanzapine was confirmed in previous studies[15], Moreover, our study proved cognitive deficits in spatial learning and memory revealed by increase in latency to reach the hidden platform in the acquisition phase in Morris water maze (MWM) in rats that are exposed to OLA as well as OLA+BET. There was also increase in latency time to enter the target quadrant and decline in time spent in it in the probe trial in the 6th day. These changes were accompanied with significant decrease in exploring the novel object significantly more than the familiar object in Social Novelty/preference Session of social interaction test (Session II).

In preclinical cognitive tests, varying results have been observed with atypical antipsychotics on normal cognitive functions. In some studies, antipsychotics disturbed cognitive functions, whereas these agents produced no effect in other studies[16].

Conclusion, from the above results we can conclude that although olanzapine had a beneficial effect on increasing sociality in isolated reared rats, it deteriorate spatial memory and novel object recognition.

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ملخص

تأثير تناول الأولانزابين على نموذج جرذان ذكور السبراج داولى لمرض الفصام الناتج عن تربية العزلة

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الفصام الذهنى هو اضطراب فى علم الأمراض النفسية يقدر بنحو ١٪ فى جميع أنحاء العالم. تربية العزلة للجرذان هو طريقة شائعة الاستخدام لنمذجة هذا المرض، وقد تم إثبات أن الأولانزابين له فعالية لعلاج مرض الفصام الذهنى بالمقارنة مع الجيل الأول من مضادات الذهان القديمة. أجريت الدراسة الحالية لاستكشاف الآثار المحتملة للاولانزابين (٦ ملج / كج / يوم) على الاجتماعية والإدراك فى الفئران التى تم تربيتها معزولة كنموذج للفصام. **وكانت أبرز النتائج:** تم إثبات أن العزلة الاجتماعية التى تم التعبير عنها بمؤشر المؤنسة قد تدهورت بشكل كبير ذو دلالة إحصائية من قبل مجموعة الفئران التى تم تربيتها معزولة وهو ما يدل على تحقق أعراض الفصام السلبية وقد أدى العلاج المزمن بدواء الاولانزابين إلى تحسن ذو دلالة إحصائية لمؤشر المؤنسة بشكل ملحوظ، وأيضا قد أدى العلاج المزمن بدواء الاولانزابين إلى ضعف ذو دلالة إحصائية فى الإدراك المشار إليه من خلال انخفاض مؤشرات التفضيل الاجتماعى، وزيادة الوقت للوصول للمنصة فى المربع المستهدف فى أول خمس أيام من اختبار المتاهة المائية لموريس ونقص الوقت المنقضى فى هذا المربع فى اليوم السادس.

الاستنتاج: كان للاولانزابين آثار مفيدة على زيادة التفاعل الاجتماعى فى الجرذان التى تم تربيتها فى عزله ولكنه قد أدى إلى ضعف الإدراك المتمثل فى الذاكرة المكانية والتعرف على الأشياء الجديدة.