OLANZAPINE AND RISPERIDONE INFLUENCE ON GESTATION AND FETAL DEVELOPMENT IN WISTAR RATS

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Abstract

There is limited data about atypical antipsychotic prescription in pregnancy because due to obvious reasons of ethical issues no randomised controlled studies can be made. Sixty pregnant female rats were divided in three groups: group A or control group (n=20), those not receiving any substance, Group B (n=20) receiving olanzapine 6 mg/kg body weight and Group C (n=20) receiving 3mg/kg body weight risperidone, by oral route. When female rats reached the 21st day of gestation they were anesthetized with halothane through gas scavenging apparatus. Cesarean dissection was done, and then the macroscopic and microscopic evaluation of uterine tissues was made. The number of live or dead embryos, the number of reabsorption and other anomalies were noted. Our experimental study revealed that olanzapine and risperidone may affect gestation in various developmental stages. The results were confirmed also by the histopatological and statistical analysis of data.

Rezumat

Siguranța utilizării antipsihoticelor atipice în sarcină este încă o controversă. Experimentele pe animale nu sunt concludente, iar din considerente etice studii clinice controlate nu pot fi efectuate. Experimentul s-a efectuat pe trei loturi de animale fiecare cuprinzând câte 20 de femele gestante de șobolani albi Wistar: lotul A (control) care au primit 0,5 mL ser fiziologic, lotul B care a primit olanzapină 3 mg/kg corp și lotul C tratat cu risperidonă, 3 mg/kg corp. Când animalele au atins vârsta gestațională de 21 zile au fost anesteziate cu halotan inhalator, iar uterul lor a fost disecat și examinat. S-a urmărit numărul fetușilor sănătoși, morți, cu anomalii, respectiv numărul de resorbții uterine. Examinarea uterină macroscopică respectiv microscopică a demonstrat faptul că administrarea olanzapinei respectiv a risperidonei la femele gestante de șobolan afectează produșii de concepție în diferite stadii ale dezvoltării intrauterine, fapt confirmat și de analiza statistică.

Keywords: olanzapine, risperidone, gestation in rats, dead fetuses.

Introduction

Women with psychiatric disorders who are pregnant or breastfeeding must continue their treatment. Olanzapine and risperidone's used during pregnancy is approved by the Food and Drug Administration (FDA) and they are the most commonly used atypical antipsychotics. But their approval was based on case reports of women receiving the drug throughout pregnancy, and there are also few preclinical studies regarding their reproductive toxicity.

The objective of the current study was to determine how olanzapine and risperidone are affecting gestation and fetal development in Wistar rats.

Materials and Methods

Sixty pregnant female rats were divided in three groups: group A or control group (n=20), those not receiving any substance, Group B (n=20) receiving olanzapine 6 mg/kg body weight and Group C receiving risperidone 3 mg/kg body weight, by oral route. The three groups were kept under the same conditions of temperature and humidity and had free access to water and food. Two males were kept with 4 females for an entire week based on rat's ovulatory cycle. Female rats have a regular 4-day oestrous cycles ovulation but ovulation can also occur spontaneously in the presence of male. After copulation the females were relocated into separate cages until parturition.

When female rats reached the 21^{st} day of gestation they were anesthetized with halothane through gas scavenging apparatus and cesarean dissection was performed. The number of live or dead embryos, the number of resorbtion and other anomalies were noted. After the uterus was removed the females were submitted to a lethal anaesthetics dose and killed.

Embryonic deaths were classified as either early (only embryonic tissue visible) or late (both the placenta and embryonic tissue visible).

Uterine tissue samples were then collected and fixed in 10 % neutral buffered formalin for histopathological examinations. The samples were embedded in paraffin wax, and were cut into 5 µm sections and stained with hematoxylin and eosin. The histological sections were examined for the presence of embryonic tissues or abnormal cells using a microscope.

The data are unpaired (independent samples). Thus, for statistical interpretation of data, descriptive statistics was performed, then a normality test was applied (Kolmogorov–Smirnov test - KS test).

Based on the results of normality test, a parametric One-Way Anova or its non-parametric equivalent (The Kruskal –Wallis – KW test) should be

chosen. Then, as a *post-hoc* test, in this case the Dunn test was applied (which compares the control *versus* experimental groups).

The statistical analyses were made using GraphPad Instat Software [1].

Animal experiments were made in concordance with Directive 2010/63/EU of the European Parliament and of the Council regarding protection of animals used for scientific purpose and approved by the Ethical Committee of University of Medicine and Pharmacy of Tîrgu Mureș, Romania.

Results and Discussion

The macroscopic and histopatological examination of uterine contents

The cesarean disection revealed 144 live fetuses in the control group. In the olanzapine group there were identified 53 resorbed embryos, 67 live fetuses and 9 dead fetuses and 52 resorbed embryos, 50 live fetuses and 2 dead fetuses in risperidone group respectively (Table I).

Table I

The influence of antipsychotics on gestation in Wistar rats

	Live fetuses	Resorbtions	Dead fetuses
Control group	144	-	-
Olanzapine group	67	53	9
Risperidone group	50	52	2

Uterine examination in olanzapine group

In a normal gestation fetuses are symmetrical distributed in the two uterine horns, as presented in Figure 1 (A). Olanzapine treated females had asymmetrical distribution of fetuses between uterine horns - Figure 1 (B).

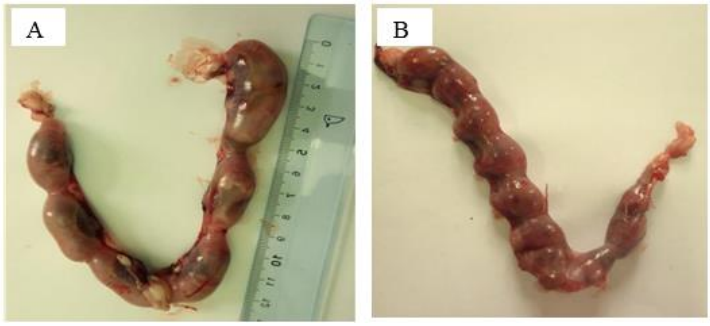

Figure 1.
Normal distribution of fetuses in uterine horns (A). Asymmetrical distribution of foetuses (B)

Resorbed embryos in various developmental stages were also seen in the experimental group (Figure 2).

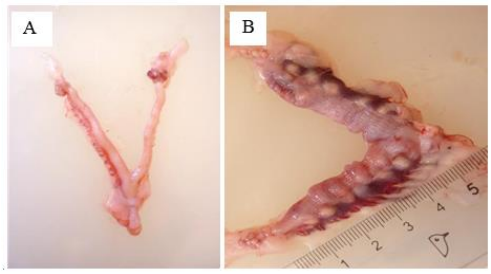

Figure 2.
Normal uterus (A) and gestating uterus with late resorbed embryos (B)

The histopathological analyses confirmed that reabsorptions occurred. Microscopic examination revealed distinct areas of embryonic tissue remnants - Figure 3.

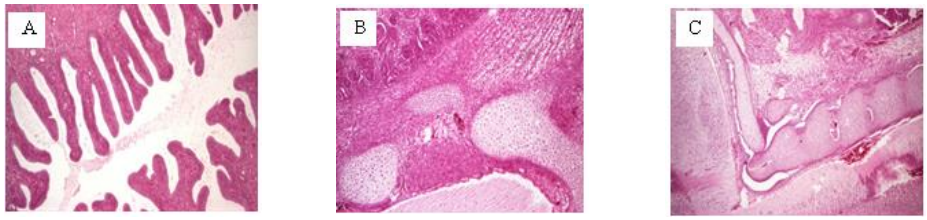

Figure 3.
Empty uterine cavity, the microscopic appearance of rat endometrium. (A). Remnants of embryonic tissue in uterine cavity (B, C). (hematoxylineosin staining, Ob.2x)

In one female rat, cesarean dissection revealed 8 fetuses, 7 healthy and alive and one of them presenting generalized edema and internal bleeding. Figure 4.

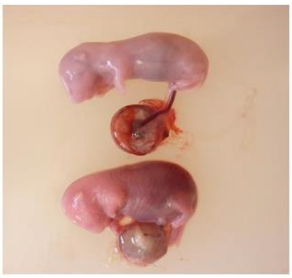

Figure 4.
Healthy, normal fetus and fetus with edema and internal bleeding

The presence of an ovular sac (Figure 5 - A), confirmed by histopathological analysis (Figure 5 - B) was identified in one female rat along with healthy fetuses as seen in Figure 2 – B.

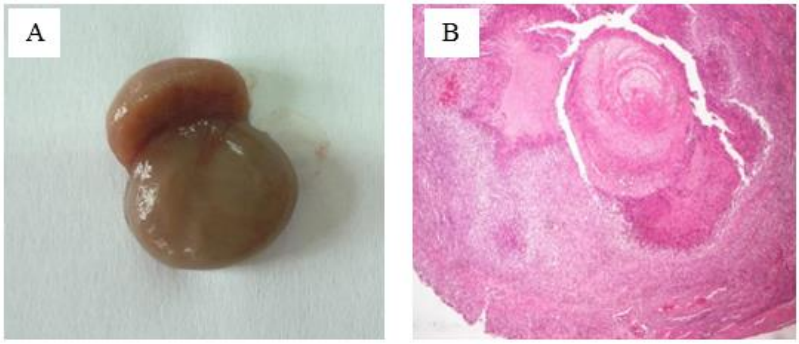

Figure 5.
Ovular sac macroscopic appearance (A) and microscopic appearance (B). (hematoxylineosin staining, Ob.2x)

Uterine examination in risperidone group

In this group early resorbed embryos were identified along with healthy fetuses, as seen in Figure 6.

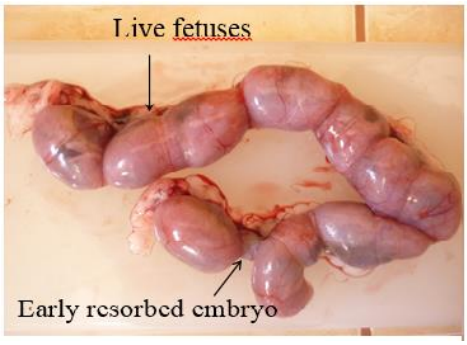

Figure 6.
Gestation affected by risperidone

The animals treated with risperidone 3 mg/kg body weight presented an increased body fat at the end of the gestation.

There were also situations in which the medication affected all the conceptus (Figure 7), placental rest and embryonic tissue remands being identified after the histopatological analysis in the uterine horns (Figure 8). As in olanzapine group, here can be seen also the asymmetrical distribution of embryos between the two uterine horns.

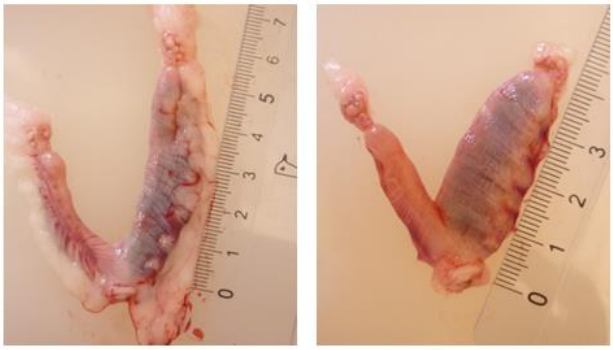

Figure 7.
Gestation affected by risperidone, with and without perivisceral fat

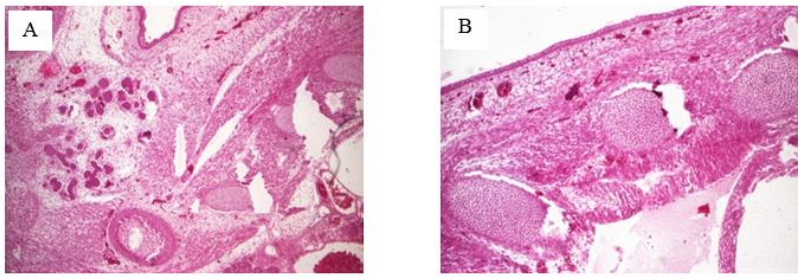

Figure 8.
Embryonic tissue remand in uterine cavity (A). (hematoxylineosin staining, Ob.2x). Embryonic tissue remand in uterine cavity (B). (hematoxylineosin staining, Ob.4x)

In one female the uteri examination revealed five conceptus of which only one developed normally, the others being dffected by the medication – Figure 9.

Figure 9.
Live fetus in yolk sac (A), live fetus outside the yolk sac (B) and live fetus with placental disc (C)

In the risperidone group it was identified also a macrosomic fetus, as seen in Figure 10.

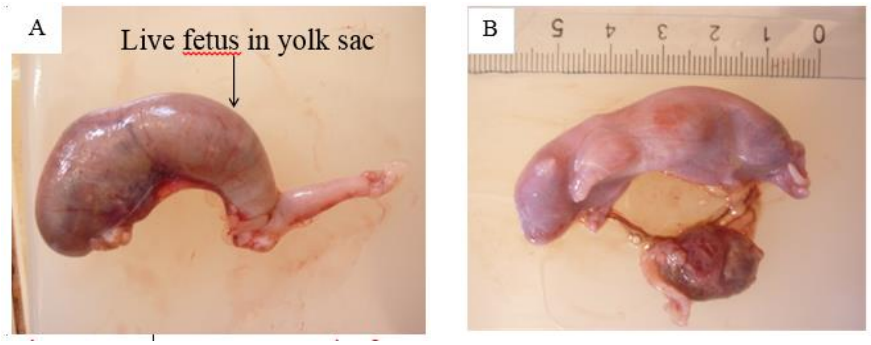

Figure 10.
Macrosomic fetus in yolk sac (A). Macrosom fetus with placental disc (B)

The statistical analysis of data

The statistical analysis was made considering the total number of accidents (resorbtions and dead fetuses), by comparing the average/mean (for a parametric test) or median (for the non-parametric test) number of resorbtions and fetal dead with the control group. The values were calculated per each female rat (total number of resorbtion or dead fetuses for each female rat).

Descriptive statistics was made, then a normality test was applied (Kolmogorov–Smirnov test - K–S test). Even though experimental data reached criteria for parametric statistical analysis, the non-parametric test of Kruskal–Wallis (K-W test) was applied because the number of the accidents in the control group was null. By using ranks instead of simple means comparisons, a non-parametrical statistical test is more suitable for a comparison with the zero values observed in the control group (where, obviously, standard deviation is also zero).

Then, the Dunn test was consequently used as *post-hoc* test, in order to compare the control with both experimental groups.

There was a statistically significant difference between medians of the number of accidents between the control group both antipsychotic drugs groups ($p<0.001$).

Conclusions

The aim of our study was to determine how olanzapine and risperidone are affecting gestation and fetal development in Wistar rats. The substances were administrated from gestation day one, because is well

known that antipsychotics can interfere with the reproductive function in rats if they are administrated before copulation. Previously reported study revealed that 5 mg/kg body weight olanzapine lengthened the estrous cycles in animals resulted in decreased mattings, longer precoital periods, and thus, fewer pregnancies [2]. Antipsychotics can have increased risk of hiper-prolactemia which can interfere with the functioning of reproductive system, including infertility; but this effect is usually seen in classic antipsychotics. The hiperprolactemia induced by classical antiphichotics can be explained by dopamine 2 (D_2) receptors blockade in the tubero-infundibular region of the brain. Atypical antipsychotics (except risperidone) are known to cause less hiperprolactinemia because of their serotonin antagonistic property of blocking 2A ($5HT_2$) receptors in addition to dopamine receptors.

Increased adiposity was identified in the treated animals compared with control group. In women, adiposity was associated with increased risk of neural tube defects. Olanzapine and clozapine are the most obesogenic and diabetogenic antipsychotics. Pregnancy itself is considered as a stressful period and may uncover impaired glucose tolerance resulting in diabetes *mellitus* [3]. This metabolic secondary effect of antipsychotics can explain the presence of the macrosomic fetus in the risperidone group. Fetal macrosomia is common in diabetic mothers, since there is an increased supply of glucose and other nutrients [4].

Newham JJ and his co-workers reported that infants exposed to atypical antipsychotics had a significantly higher incidence of large for gestational age (LGA) [5]. A large for gestational age (LGA) infant was delivered also by o women diagnosed woman in her third trimester with delusional and borderline personality disorder and successfully treated with olanzapine [6]. There were also tendencies to low birth weight among neonates exposed to olanzapine [7]. The increased risk of giving birth to a small for gestational age infant after antipsychotics exposure was also suggested by Bodén R *et al*, 2012. But except for macrocephaly, olanzapine or clozapine exposure was not associated with anabolic fetal growth in 169 women [8].

In our study olanzapine and risperidone affected gestation in various developmental stages. We identified ovular sac, early resorbed embryos and late resorbed embryos. The presence of placenta and embryonic tissues remnants in uterine horns were confirmed by the histopathological analysis.

The statistical analysis confirmed that the fetal development in rats was affected in animals exposed to olanzapine or risperidone during gestation period. A statistically significant difference has been observed

between the median number of accidents between the control group and olanzapine and risperidone group, respectively.

Antipsychotics exposure during pregnancy was not associated with the risk of major malformation in women but our animal study revealed that in rat olanzapine may affect gestation in various developmental stages.

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