

TIME AND DOSE-RELATED SIDE EFFECTS OF RISPERIDONE IN RATS

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Abstract

Although it is included in the modern antipsychotics group, risperidone distinguishes itself from the others through its particular pharmacotoxicological profile. The study aimed to identify the main pharmacotoxicological targets located at different organs levels, by using a 9-week chronic treatment experimental model designed on white Wistar rats. 60 animals were assigned in groups of 10 individuals, as follows: group 1 (males) were treated with 1mg/kg bw risperidone, groups 2, 3 and 4 (females) were treated with 1, 2 and 4 mg/kg bw risperidone respectively, while group 5 and group 6 served as controls (males and females respectively). Blood and tissue samples (mammary gland, fatty tissue) were taken after the sacrifice of the animals, in order to determine 25-hydroxyvitamin-D concentration in parallel with the histopathological processing and its assessment respectively. The chronic treatment with risperidone produced fatty tissue hypertrophy, reflected in body weight gain, hyperprolactinaemia (secreting glandular acini), important vitamin D plasma levels decreases, without any radiologically noticeable skeletal alterations. Hyperprolactinaemia and fatty tissue hypertrophy were shown to be present in the chronic risperidone treatment in rats, but the bone alterations are discreet, indicated only indirectly by the significant lowering of plasma vitamin D levels.

Rezumat

Risperidona, inclusă în grupa antipsihoticelor noi, se diferențiază printr-un profil farmacotoxicologic particular. Studiul urmărește identificarea principalelor ținte farmacotoxicologice la nivel de organ, într-un model experimental la șobolani albi Wistar în tratament cronic (9 săptămâni). 60 de animale au fost repartizate câte 10/lot astfel: lotul 1 (masculi) tratați cu risperidonă 1 mg/kg corp, lotul 2, 3, 4 (femele) cu 1, 2, respectiv 4 mg/kgc, iar loturile 5, 6 au servit drept control (masculi/femele). S-au prelevat mostre biologice după sacrificare (glanda mamară, țesut adipos, sânge) pentru prelucrare histopatologică, în paralel cu determinarea concentrației 25-hidroxivitaminăi-D. În tratament cronic, risperidona produce hipertrofia țesutului adipos reflectată prin creștere ponderală, hiperprolactinemie (acini glandulari în secreție), scăderea semnificativă a concentrației vitaminei D plasmatică, fără modificări ale scheletului, evidențiabile radiologic. Hiperprolactinemia și hipertrofia țesutului adipos sunt prezente în tratament cronic cu risperidonă la șobolan, dar modificările osoase sunt discrete, neevidențiabile decât prin scăderea semnificativă a nivelului plasmatic de vitamină D.

Keywords: risperidone, hyperprolactinaemia, bone damage

Introduction

Atypical antipsychotics like olanzapine, clozapine and risperidone have higher affinity for serotonin 5-HT₂ receptors compared to dopamine D₂ receptors (the affinity ratio for the two receptor types was shown to be around 10:1) [1]. There are also some hypotheses that D₄ receptors or D₂ and D₄ receptors altogether are antagonized by atypical antipsychotics, or the antagonists rapidly dissociate from the receptors after the blockade [7].

Risperidone is a polyvalent neuroleptic that has higher affinity for serotonin (5-HT) receptors, being effective on the positive symptoms of schizophrenia, but mainly on the negative ones.

Blockade of the following serotonin receptor subtypes is considered to be mainly involved in the antipsychotic mechanism of action: 5-HT_{2A}, 5-HT_{1A}, 5-HT₆, 5-HT₇ and 5-HT_{2C}, therefore atypical antipsychotics present fewer side effects of extrapyramidal origin compared with classic antipsychotics. Almost all atypical antipsychotics are 5-HT_{2A} receptor inverse antagonists and dopaminergic (D₂) antagonists or partial agonists [1].

The antagonistic action on 5-HT_{2C} serotonin receptors is responsible for the metabolic side effects of atypical antipsychotics, while the agonistic activity helps to improve the cognitive performance [8].

As the data reported in the literature with regards to the side effects of risperidone are very controversial, we aimed to evaluate risperidone's side effects at different doses in Wistar rats.

Materials and Methods

The experiment was performed using 6 animal groups of white Wistar rats, separated by gender (n=10 /group). Animals aged 30 weeks, with body weights between 260 and 320 grams, were selected for the experiment, and *ad libitum* access to food

and water, as well as a light/dark cycle of 12 hours were constantly provided.

For a period of 9 weeks, the experimental groups were treated as follows: males received 1 mg/kg bw risperidone daily, and the female groups received 1mg/kg bw, 2 mg/kg bw or 4 mg/kg bw risperidone daily respectively. Two additional groups, one of each gender, served as control groups and only received the vehicle used for suspending the active substance, as presented in Table I.

Table I
The distribution of animals in groups

Group	No. of animals / gender	Medication
1.	10 white Wistar rat males	1 mg/kg bw risperidone
2.	10 white Wistar rat females	1 mg/kg bw risperidone
3.	10 white Wistar rat females	2 mg/kg bw risperidone
4.	10 white Wistar rat females	4 mg/kg bw risperidone
5.	10 white Wistar rat males	Vehicle – without active substance
6.	10 white Wistar rat females	Vehicle – without active substance

At the end of the experiment, animals were sacrificed using phenobarbital 50 mg/kg bw i.p. injection, then mammary gland tissue and blood samples were collected. Tissue samples were processed for histological examination, and 25-hydroxy-D-vitamin was determined from plasma. The blood samples were collected on K₃EDTA anticoagulant, then centrifuged at 3500 rpm and 4°C, and the plasma was subsequently stored at -20°C until the assay. The analyses were performed by a certified laboratory, using an isocratic validated HPLC with UV detection method, using protein precipitation and solid phase extraction analyte concentration techniques (according to the specifications of the producer Chromosystems). The reported results were compared with the ones obtained in the control groups.

Before being sacrificed, the animals were sedated with medetomidine 0.4 mg/kg bw so that a radiological examination with latero-lateral exposure could be done using a veterinary Univet LX 120 radiology system, with parameters set at 40 kV and 5 mAs.

Breast 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 haematoxylin and eosin. The histological sections were examined with a microscope.

For the entire duration of the experiment, the animals were housed at the Târgu Mureş University of Medicine and Pharmacy's Biobase, and were provided free access to water and standard diet.

Animal experiments were performed according to the 2010/63/EU Directive of the European Parliament and European Council regarding the protection of animals used for scientific purposes, and were approved by the Ethics Committee (Institutional Review Board) of the University of Medicine and Pharmacy of Târgu Mureş.

Statistical analysis

All data are reported as mean ± SD. The data were first analysed for detecting outliers using Grubbs' test. Each subset was tested for normal distribution with Kolmogorov-Smirnov test. Differences between groups were analysed using the nonparametric ANOVA – Kruskal-Wallis test or Friedman test (for repeated measures) with Dunn's Multiple Comparison *post hoc* test, and One-way ANOVA test with Tukey's Multiple Comparison *post hoc* test. Statistical significance limit was set at $p < 0.05$.

Results and Discussion

Body weight variations

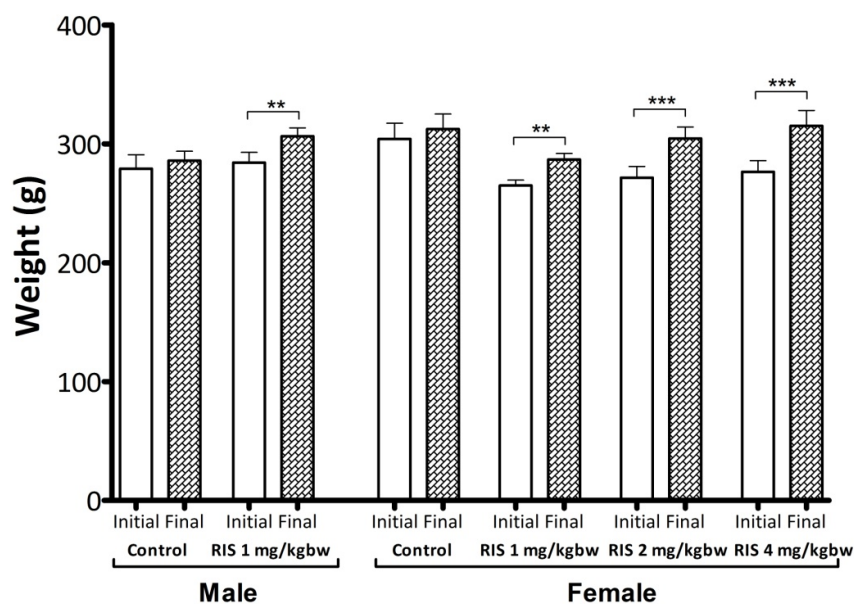
The body weights of the animals were recorded weekly during the treatment. Influences of risperidone treatment on rats' body weight variations were observed. A low dose of 1 mg/kg bw risperidone increased the body weight in both genders after nine weeks of treatment. Dose dependency of this effect was investigated in female rats. Higher doses of risperidone – 2 mg/kg bw and 4 mg/kg bw respectively – caused weight gain in a dose-dependent manner (Table II, Figure 1).

Table II

Body weight variation in the studied animal groups

Gender	Groups	Mean difference (95% CI) between final and initial weight (g)	Friedman statistic	p value
Male	Control	6.67 (1.09-12.25)	32.97	< 0.001*
	Risperidone 1 mg/kg bw	22.17 (12.77-31.56)	50.50	< 0.001
Female	Control	8.33 (5.62-11.04)	31.84	< 0.001*
	Risperidone 1 mg/kg bw	21.83 (16.46-27.20)	52.55	< 0.001
	Risperidone 2 mg/kg bw	33.00 (28.17-37.83)	88.04	< 0.0001
	Risperidone 4 mg/kg bw	38.50 (35.11-41.89)	89.38	< 0.0001

* No statistically significant difference was observed between initial weight and the weight of the animals during the treatment period using *post-hoc* Dunn's Multiple Comparison test.

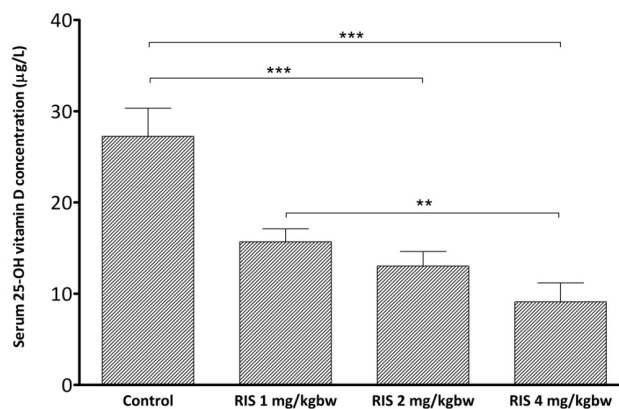
**Figure 1.**

Body weight of the animals determined before and after the treatment

Legend: ** - $p < 0.01$, *** - $p < 0.001$, p values were calculated with Friedman test and Dunn's Multiple Comparison test; RIS – risperidone.

Literature data suggest that long term risperidone treatment induces bone damage [4], therefore we aimed to investigate if this side effect is related to blood 25-hydroxy-D-vitamin concentration decrease. Serum 25-OH vitamin D concentrations were measured after risperidone treatment, and showed

significant differences between groups (KW statistic = 34.28, $p < 0.001$). Risperidone decreased the serum concentration of 25-OH vitamin D in a dose-dependent manner (Figure 2).

**Figure 2.**

Serum concentration of 25-OH vitamin D in the female animal groups

Bone damage was radiologically investigated as presented in Figure 3 and 4.

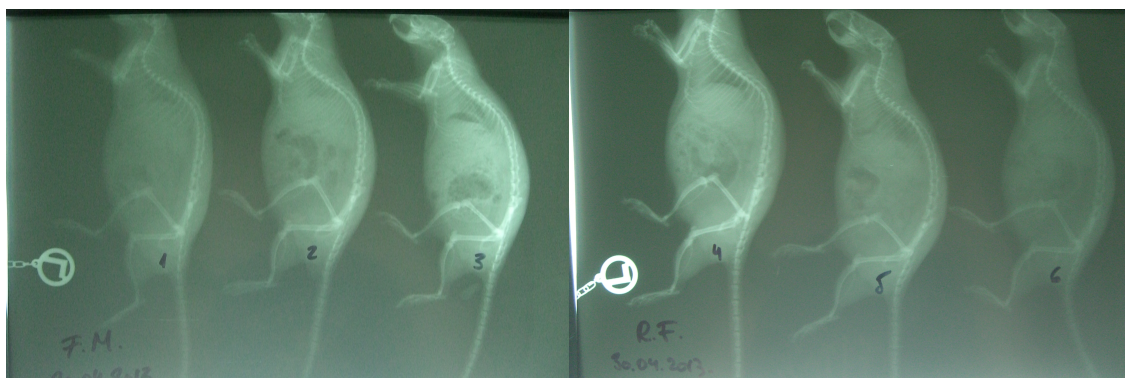


Figure 3.

Control group females

Figure 4.

Risperidone treated group (4 mg/kg bw)

There were no significant radiological differences between the control group and risperidone treated groups, even at the higher administered doses. The observation of radiological results was performed by a specialist.

The *hyperprolactinemia* induced by risperidone treatment was histopathologically confirmed by the presence of milk drops in breast glandular acini and galactophore channels.

Active mammary glands were identified in the risperidone treated animals, as compared to the control group animals, in which non-lactating mammary glands have been noticed, with the alveoli and the terminal duct system was surrounded by connective tissue (Figure 5). The alveoli were distended and partially filled with milk. (Figure 6, 7 and 8).

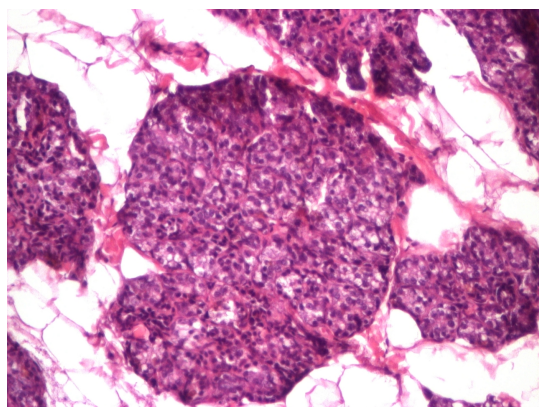


Figure 5.

Mammary gland in the control group (HE, 10X)

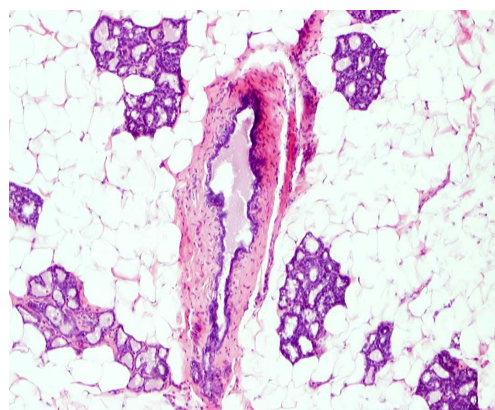


Figure 6.

Teat canal with milk secretion, in the risperidone treated group – 1 mg/kg bw (HE, 10X)

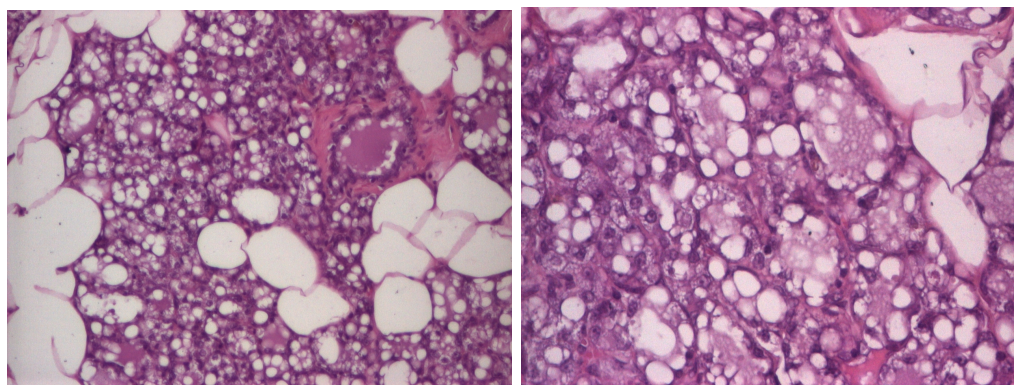


Figure 7.

Active mammary gland, in the risperidone treated group – 1 mg/kg bw (HE, 20X)

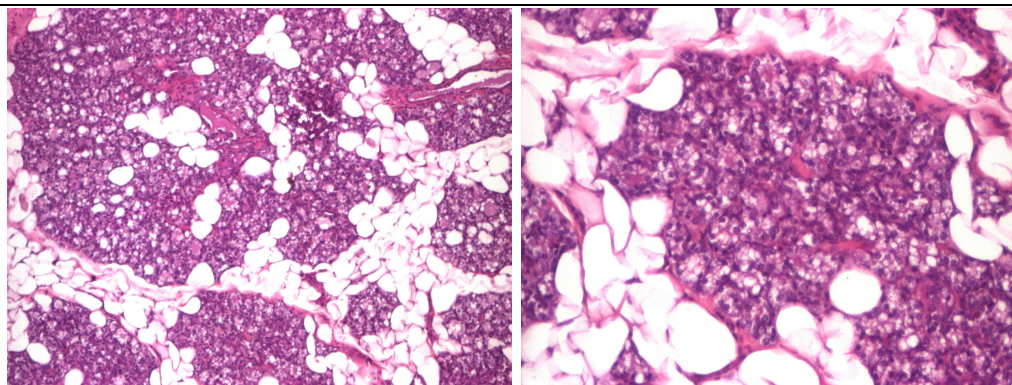


Figure 8.

Active mammary gland in the risperidone treated group – 2 mg/kg bw (HE, 20X)

From day 14-21 of treatment *behavioural changes* could be noted in risperidone treated animals, especially in females, which started nest building. Risperidone treatment was associated with body weight increase and metabolic side effects. Although the mechanisms responsible for these side effects are not fully understood, it is supposed that the antagonistic effect of risperidone on 5-HT_{2C} receptors from POMC (proopiomelanocortine) neurons is responsible for the orexigenic effect [1, 6]. There are two hormones that play an important role in the regulation of food intake, energy metabolism, and body weight, namely leptin and ghrelin. Leptin is a mediator of long-term regulation of energy balance, suppressing food intake and thereby inducing weight loss (Mohamad *et al.*). Ghrelin on the other hand is a fast-acting hormone, seemingly playing a role in meal initiation. In a study made on 112 patients treated either with clozapine (n = 20), olanzapine (n = 28), risperidone (n = 22), quetiapine (n = 20) or amisulpride (n = 22) as monotherapy for at least one year, who had their age, gender, and body mass index (BMI) matched with the control group (n = 23), the subjects were assessed cross-sectionally for ghrelin and leptin levels, which were measured using enzyme-immunoassays. The authors concluded that the weight-gain side effect of the atypical antipsychotics can be related to the orexigenic effect of elevated serum ghrelin rather than leptin deficit [3, 4, 12].

Vestri *et al.*, 2007, examined the hypothesis that second-generation antipsychotics have direct cellular effects on insulin action and substrate metabolism in adipocytes, which is associated with weight gain and the development of diabetes mellitus. The experiments made on two cultures cells including 3T3-L1 adipocytes and primary cultured rat adipocytes have demonstrated that the antipsychotic drugs can differentially affect insulin action and metabolism through direct cellular effects in adipocytes. Risperidone reduced insulin-stimulated glucose transport rates in primary

cultured rat adipocytes by approximately 50-70%, such effects on adipocytes partially explaining the association of second-generation antipsychotics with weight gain and diabetes [2, 5, 11].

In our study, a decrease in body weight was noted after the first week of treatment, then the animals started to gain weight until the end of the experiment, when the registered results statistically increased compared to the initial measurements, in all risperidone treated groups.

Studies in women with hyperprolactinemia induced by pituitary tumors have demonstrated high rates of osteoporosis, believed to be the result of related hypoestrogenism. Similarly, hyperprolactinemia in men results in hypogonadism and bone loss. Preliminary surveys have indicated that schizophrenic patients may also have elevated rates of osteoporosis and pathological fractures, possibly resulting in part from the long-term administration of antipsychotic agents that produce hyperprolactinemia and secondarily lower oestrogen and testosterone levels [10, 15].

Motyl KJ *et al.*, 2012 studied risperidone's (1.25 mg/kg bw daily) effect on bone metabolism using an *in vivo* and *in vitro* model of C57BL6/J (B6) mice. Risperidone reduced trabecular BV/TV, trabecular number and percent of cortical area. Trabecular histomorphometry demonstrated increased resorption parameters, with no change in osteoblast number or function. The number of osteoclasts was significantly increased by the *in vitro* addition of risperidone, while osteoblast differentiation was not altered [9].

In our study the hyperprolactinemia induced by risperidone is obvious even at small doses, when compared with the control group. Although there were no radiological differences between the control groups and risperidone treated groups, the decrease in 25-OH vitamin D plasma levels of the latter confirmed that the medication might cause bone damage.

Previous study reports suggest that atypical antipsychotics disrupt active components of

maternal behaviour such as pup approach, pup retrieval and nest building at clinically relevant doses in postpartum female rats, primarily by blocking the 5-HT(2A/2C) receptors [13].

The risperidone treated female rats included in our study were noted for engaging in nest building behaviour from day 14-21 of the study, although they were not pregnant at the time, fact that could be also explained by serotonin receptors blockade.

Conclusions

The presented study showed that a 9-week risperidone treatment induced significant weight gain in rats, which was related to metabolic disturbances (i.e. the increase of visceral fatty mass, hyperprolactinemia and plasma 25-hydroxy-vitamin-D decrease). The hyperprolactinemia and the decrease in plasma 25-hydroxy-vitamin-D could lead to bone damage, although no radiological differences were noted in the animals treated with risperidone compared to the control group.

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