Int. J. Devl Neuroscience 52 (2016) 13–23

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Effects of prenatal exposure to antipsychotic risperidone on developmental neurotoxicity, apoptotic neurodegeneration and neurobehavioral sequelae in rat offspring

K.P. Singh [a,*], Manoj Kr. Singh [a], Manish Singh [b]

[a] Neurobiology Lab., Department of Zoology, University of Allahabad, Allahabad 211 002, India
[b] Institute of Nanoscience and Technology, Mohali, India

ARTICLE INFO

Article history:
Received 12 March 2016
Received in revised form 6 May 2016
Accepted 12 May 2016
Available online 13 May 2016

Keywords:
Antipsychotic
Risperidone
Prenatal
Fetal neocortex
Apoptosis
Neurobehaviour
Postnatal growth
Rat

ABSTRACT

A tremendous increase has been documented in the recent past in prescribing second generation atypical antipsychotic drugs (AAPDs) to the pregnant women with psychosis, considering their reproductive and teratogenic safety. Among AAPDs, risperidone (RIS) ranked third after olanzapine (OLZ) and quetiapine (QUE) used during pregnancy, as OLZ is associated to substantial weight gain in adults and offspring. Although teratogenic safety of RIS has been established, its potential role in developmental neurotoxicity and related neurobehavioral impairments in adolescents has not been documented so far. Therefore, present study has been undertaken to elucidate the effect of prenatal exposure to risperidone (RIS) on developmental neurotoxicity and apoptotic neurodegeneration in neocortical region of fetal brain; and related functional sequelae in young rat offspring. The pregnant Wistar rats were exposed to RIS at 0.8, 1.0 and 2.0 mg/kg, at equivalent therapeutic doses, orally from GD 6 to 21. Half of the pregnant rats were sacrificed and their brains were collected, weighed, and processed for neurohistopathological and apoptotic neurodegenerative evaluation. The remaining dams were allowed to deliver naturally, and their offspring were reared up to 10 weeks for neurobehavioral study. Prenatal exposure to RIS induced significant stunting of fetal body and brain weight, substantial reduction in the thickness of neocortical layers and apoptotic neurodegeneration in fetal brains, and delayed postnatal development and growth of the offspring; as well as long- lasting impact on anxiety like impaired behavioral responses on explorative mazes. Therefore, health care providers should be careful in prescribing atypical antipsychotics in general and RIS in particular, to the pregnant psychotic population.

1. Introduction

Several second generation novel or atypical antipsychotic drugs (AAPDs) are available in the global market for pharmacotherapeutic intervention of different forms of psychotic illness including schizophrenia and bipolar disorder in adult population.

The reproductive and teratogenic safety of AAPDs has been improved to the great extent in comparison to the classical antipsychotic drugs (APDs) like haloperidol; consequently, a tremendous increase has been observed in prescribing AAPDs to the pregnant population (Galbally et al., 2014; Stephenson et al., 2013; Toh et al., 2013). Among second generation AAPDs, risperidone (RIS) ranked third after olanzapine (OLZ) and quetiapine (QUE) used during pregnancy (Paschetta et al., 2014; Sadowski et al., 2013). Although AAPDs are believed to be safer than typical APDs, but the side effects associated with metabolic dysregulation, weight gain in pregnant subjects, and low or heavier birth weight babies with poor neonatal adaptation signs; have also been observed (Habermann et al., 2013; Sadowski et al., 2013).

Risperidone (RIS), a benzisoxazole derivative, is principally used to treat serious psychiatric illness like schizophrenia, schizoaffective disorders, bipolar disorder, and irritability in people with autism (Abel and Howard, 2014; Risperdal Product Monograph, 2005). It is unique among other AAPDs, since it has milder, but still potent affinity for the D_2 receptor, whereas others have 'loose binding' of the D_2 receptor (Leysen et al., 1994). It is a dopamine (D_2, D_3) receptor antagonist, possessing antiserotonergic (5-HT_{2A}, 5-HT_{2C}), antiadregenic (α_1, α_2), antihistaminergic (H_1) properties. It has more prominent serotonin antagonism than dopamine

Abbreviations: RIS, risperidone; APD(s), antipsychotic drug(s); AAPD(s), atypical antipsychotic drug(s); GD, gestation day; MRHD, maximum recommended human dose; DCFDA, 2,7-dichloro fluorescein diacetate; ROS, reactive oxygen species; PS, phosphatidyl serine; FITC, fluorescein isothiocyanate; PI, propidium iodide; Bcl-2, B-cell lymphoma-2 protein (anti-apoptotic); Bax, Bcl-2-associated X protein (pro-apoptotic); 5HT, serotonin; OFT, open-field test.

* Corresponding author.
E-mail address: kpsald29@gmail.com (K.P. Singh).

http://dx.doi.org/10.1016/j.ijdevneu.2016.05.006
0736-5748/

antagonism (Risperdal Product Monograph, 2005). It has intense affinities with 5-HT (serotonin) receptor sub types as 5-HT_{2C} linked to weight gain, 5-HT_{2A} linked to its antipsychotic action and relief from extra-pyramidal side effects. The molecular weight of RIS is 410.49 g/mol; hence it can easily cross the placental and blood- brain barriers effectively controlling the psychotic illness (Risperdal Product Monograph, 2005).

There is a paucity of clinical and non- clinical studies on developmental neurotoxicity caused by maternal exposure to AAPDs in general and RIS, in particular. Also, no study was found on neurohistopathological changes in fetal brain caused by *in utero* exposure to RIS. This laboratory has already reported the developmental neurotoxic potential of some CNS acting drugs like antipsychotics, quetiapine and aripriprazole (Singh and Tripathi, 2015, 2014); antidepressants, venlafaxine (Singh et al., 2015); antiepileptics, gabapentin and sodium valproate (Singh and Gupta, 2014) and their effects on developing brain. Some investigators have revealed the effect of typical antipsychotics like haloperidol (HAL) on developmental structural changes in fetal brain whose mothers were administered at 1st/2nd and/or 3rd trimester(s) of pregnancy. Similar studies were also reported in rodent model (Singh and Singh, 2001, 2002; Zhang et al., 1996). However, limited information is available on effects of *in utero* administration to AAPDs including RIS on postnatal development and growth; and neurobehavioral consequences in young/adult rodent offspring (Zuo et al., 2008; Karl et al., 2006; Rosengarten and Quartermain, 2002). A few clinical studies (Galbally et al., 2014) have reported an association between *in utero* exposure to AAPDs and delayed neuromotor/neurodevelopment, delayed cognitive and adaptive behaviour in infants (Peng et al., 2013; Johnson et al., 2012). Thus, there is scarcity of data on vital issue of neural health in the developing fetal brain, hence, need to be investigated.

Therefore, considering extreme paucity of existing knowledge on developmental neurotoxicity of RIS in clinical and preclinical settings (rodent models), present study has been undertaken to elucidate substantive information on effects of prenatal exposure to RIS, at equivalent therapeutic doses, during critical period of brain development, on developmental neurotoxicity (cytoarchitecture of neocortex and apoptotic neurodegeneration) in fetal brain, postnatal development and growth (body weight gain); and neurobehavioral (anxiety like) sequelae in young rat offspring, as long-lasting impact of the drug.

2. Material and methods

2.1. Animals

Laboratory inbred nulliparous female Wistar rats from the breeding colony, weighing 180 ± 10 g, were used for the experimental procedures. Animals were housed in plastic cages with rice bran as bedding material at standard laboratory environment (24 ± 2 °C, 12/12 h light/dark cycle and 60% RH). Palleted animal (rat/mice) food and tap water were made available ad libitum. Animals were maintained and used in accordance with the Animal Welfare Act and the protocol for use of experimental rats was approved by Institutional Animal Ethics Committee (IAEC), University of Allahabad, Allahabad, India.

2.2. Determination of pregnancy

After acclimatization, female rats were first allowed to mate with male overnight (ratio 2:1) and in the next morning, they were checked for the presence of sperms in vaginal smear for determining the onset of gestation. Such sperm positive rats were designated as gestation day o (GD-0) as per Vorhees et al. (2012).

2.3. Experimental design, drug exposure and rationale for selection of doses

The drug, RIS was procured from the pharmaceutical market with trade name Risperdal (Torrent, India). The maximum recommended human dose (MRHD) of RIS is 04–12 mg/day for adults. The experimental doses of the drug were calculated as 0.8 mg/kg (4 × MRHD), 1.0 mg/kg (6 × MRHD), and 2.0 mg/kg (10 × MRHD), considering the therapeutic doses on the basis of 'per kg body weight per day', and it's suitability to the animal model. The rationale for selection of three doses of RIS was as per MRHD; and higher metabolic rate of rats 4–6 times faster than in humans (Kapur et al., 2003). Four groups of pregnant female rats containing twelve rats (n = 12) per group were maintained. All the control and experiment rats were exposed to RIS or vehicle from gestational day 6–21 (GD 6–21). The selected doses of RIS (0.8, 1.0, and 2.0 mg) were prepared daily before exposure. Each tablet which contains 2 mg/kg of RIS was dissolved in 0.1 N HCL with water and calibrated according to body weight of each subject, and gavaged to sperm positive dams once daily (at 9.00 h) from GD6-21, orally with the help of cannula. Animals were primed for gavaged with sucrose water to avoid any handling stress during drug delivery. Equivalent volume of the vehicle (0.1 N HCL with water) was also given to control subjects through same route and time from GD6 to 21. As per our laboratory protocol, half of the control and RIS treated rats of each group (n = 6/group) were sacrificed under phenobarbital anaesthesia on GD 21(06.00 h), and their fetuses were collected through uterectomy. The remaining half of the dams of each group (n = 6/group) were survived and allowed to deliver naturally, and neonates/pups were further reared for neurobehavioral observations.

2.4. Measurements of fetal brain size and weight

Among the fetal pool of each group, two fetuses from each dam of control and drug treated groups were randomly selected and their brains (n = 12 brains/group) were dissected immediately, weighed and then fixed in 10% neutral formaldehyde for histopathological observation.

2.5. Measurement of thickness of neocortical layers and histopathological observations

The prefixed fetal brains were processed for histopathology as per standard protocol. In brief, fetal brains were thoroughly washed, dehydrated in ascending grades of alcohol, incubated in molten paraffin wax (58 °C) and finally embedded in paraffin blocks. After trimming, serial sections were cut at 7 μm by rotary microtome and transferred on egg albumin coated on glass slides; and finally stained with crysal violet or H&E. For measurement of neocortical thickness, five alternate sections from each brain per dams (n = 30 sections/group) were selected at mid dorsal region of the neocortex. The relevant neocortical area in coronal brain sections of control and RIS exposed fetuses were identified with the help of Atlas of prenatal rat brain development (Altman and Bayer, 1995); and imaged under Eclipse CCD camera of Nikon 83I light microscope. These photomicrographs were used for measuring the thickness of the different neuronal layers of neocortex by using Image-J software (1.46r).

2.6. In situ detection of apoptosis by confocal microscopy

Immediately after uterectomy, fetuses were anesthetized and perfused transcardially with PBS followed by fixative 2.5% glutaraldehyde & 2% paraformaldehyde, in 0.1 M phosphate buffer (pH 7.4). The brains were quickly removed from cranium, and further fixed in the same fixative for confocal microscopy. As