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In utero exposure to atypical antipsychotic drug, risperidone: Effects on fetal neurotoxicity in hippocampal region and cognitive impairment in rat offspring

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ABSTRACT

Clinical studies indicate that about one-third of pregnant women with psychotic symptoms are exposed to either typical or atypical antipsychotic drugs (APDs). Reports on prenatal subject/model are lacking hence, the present study was undertaken to investigate the effect of prenatal exposure to risperidone (RIS) on the fetal hippocampus, and their related functional changes in young rat offspring. In this study, pregnant Wistar rats were exposed to equivalent therapeutic doses of RIS at 0.8 mg/kg, 1.0 mg/kg, and 2.0 mg/kg BW from gestation days (GD) 6 to 20. On GD 21, about half of the pregnant subjects of each group were euthanized, their fetuses were collected, fetal brains dissected, and processed for neurohistopathological evaluation. Remaining pregnant dams were allowed to deliver naturally and reared up to 8 weeks of age for neurobehavioral study under selected paradigms of cognition. Our results indicate that there was a significant decrease in the thickness of fetal hippocampus with the disturbed cytoarchitectural pattern, and volume of striatum and choroid plexus was also reduced. Furthermore, RIS treated young rat offspring displayed memory impairment on different mazes of learning and memory. The current study concludes that maternal exposure to clinically relevant doses of RIS may induce neurostructural changes in developing hippocampus and striatum, and cognitive sequelae in young offspring, respectively. Therefore, caution must be taken before prescribing this drug to pregnant subjects, especially during the sensitive phase of brain development. Hence, clinical correlation of animal data is urgently warranted.

1. Introduction

The health care providers always have a dilemma for therapeutic management of pregnant women suffering from different forms of psychosis like schizophrenia and other psychiatric disorders, considering the safety concerns for diseased mother and developing fetus, concurrently. Hence, there is a need to select a suitable antipsychotic drug (APD); and decide the treatment regime and exposure period, since several antipsychotic agents belonging to diverse classes like the first generation (classical or typical), second generation (novel or atypical) and third generation (atypical) antipsychotics. The general safety concerns of these APDs have been improved enormously from development of classical APDs to novel APDs, considering their efficacy, pharmacokinetics and pharmacodynamic; and interaction with other drugs of this class. However, none of these drugs have been found to be safer for the treatment of pregnant population suffering from psychosis (Newharm et al., 2008; McKenna et al., 2005; Coppola et al., 2007). It is well documented that first-generation APDs are associated with typical side effects like extrapyramidal syndrome (EPS) and movement disorder, whereas second generation APDs are coupled with metabolic dysregulation, especially the weight gain. The reproductive and teratogenic safety of first generation APDs has been well established in clinical and non-clinical studies, however, their safety measures are yet to be ascertained in atypical agents. Hence, selection of second generation APDs among all available atypical agents is an alternate option to treat the pregnant women after weighing the balance between potential benefits to pregnant mother and risks to developing fetus, respectively. Recently, a tremendous increase has been observed in prescribing atypical/novel APDs to the pregnant population (Galbally et al., 2014; Stephenson et al., 2013; Toh et al., 2013). Epidemiological studies documented that use of atypical antipsychotic drugs (AAPDs) has been increased from 16.6 to 51.2% during 1998 to 2007 (Prah et al., 2012). Among the second generation antipsychotic drugs (SGAPDs), olanzapine (OLZ) ranked first, followed by quetiapine (QUE) and risperidone (RIS) for their use during pregnancy (Paschetta et al., 2014; Sadowski et al., 2013). clinical literature revealed that OLZ is associated with excessive weight gain in schizophrenic patients with prolonged therapy (Lambert et al., 2005; Duggan et al., 2005), whereas QUE may cause mild body weight gain (Brecher et al., 2000;

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Shaw et al., 2001), and RIS designated as neutral in weight gain (Singh et al., 2014). Hence, RIS could be a better alternative for pregnant women with psychosis.

Although, atypical APDs are supposed to be safer than typical APDs in respect to teratogenic and metabolic safety, but their neurodevelopmental and psychopathological safety of novel APDs has not been established so far in general and RIS, in particular. The clinical and non-clinical studies on these issues are very limited (Newport et al., 2007; McKenna et al., 2005; Coppola et al., 2007). Limited information is available on neurobehavioural consequences (cognitive impairment) in rodents to draw a definite conclusion (Green et al., 2002; Rosengarten and Quartermain, 2002; Karl et al., 2006; Zuo et al., 2008). Reports on developmental neurotoxicity after prenatal exposure to RIS are almost negligible. Recently, our laboratory has reported developmental neurotoxic and neurobehavioural potential of quetiapine and aripiprazole in fetuses and young rat offspring, respectively (Singh and Tripathi, 2014, 2015).

Risperidone (RIS), a benzisoxazole derivative, is principally used to treat a serious psychiatric illness like schizophrenia, schizo-affective 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, a potent affinity for the D_2 receptor, whereas others have 'loose binding' of the D_2 receptor (Leysen et al., 1988). It is a dopamine (D_2, D_3) receptor antagonist, possessing antiserotonergic (5-$HT_{2A,}$ 5-HT_{2C}), antiandrogenic ($\alpha_{1,}$ α_2), antihistaminic (H_1) properties. It has more prominent serotonin antagonism than dopamine antagonism (Risperdal Product Monograph, 2005). It has intense affinities with 5-HT (serotonin) receptor sub-types as 5-HT_{2C} is linked to weight gain, 5-HT_{2A} linked to its antipsychotic action and relief from extrapyramidal side effects. The molecular weight of RIS is 410.49 g/mol, hence it can easily cross the placental and blood-brain barriers, thus effectively controlling the psychotic illness (Risperdal Product Monograph, 2005).

Due to better efficacy and improved pharmacokinetic and pharmacodynamic properties of RIS in respect to *in utero* exposure and its safety concerns as well as paucity of literature on fetal brain development and long-lasting impact on neurobehavioral consequences, present study has been undertaken to elucidate the effects of *in utero* exposure to RIS, at equivalent therapeutic doses, on developmental neurotoxicity (cytoarchitectural pattern) of hippocampus, striatum and choroid plexus in fetal brain; and psychopathology (cognitive impairments) in young rat offspring, as translational approach.

2. Material and methods

2.1. Animals

In the present study, laboratory inbred female Wistar rats, 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). Standard pelleted rat food and purified 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 (Singh et al., 2016a).

2.2. Determination of pregnancy

After acclimatization, female rats were first allowed to mate with a 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 with gestation day 0 (GD 0) as per Vorhees et al., 2012.

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

As previously described (Singh et al., 2016a), 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 from gestational day 6 to 21 (GD 6–21), a sensitive and critical phase of fetal brain development (Costa et al., 2004), either to the drug (doses of RIS) or vehicle. In this study, selected doses of RIS (0.8, 1.0, and 2.0 mg) were prepared daily before exposure. Each tablet containing 2 mg/kg of RIS was dissolved in 0.1 N aqueous HCL and calibrated according to the body weight of each subject, and gavaged to sperm positive dams once daily (at 9.00 h) from GD 6–21, orally with the help of cannula. Animals were primed for gavaged with sucrose water to avoid any handling stress during drug delivery. An equivalent volume of the vehicle (0.1 N aqueous HCl) was also given to control subjects through same route and time from GD 6 to 21. As per our laboratory protocol, half of the control and RIS-treated rats of each group ($n = 6$/group) were euthanized under phenobarbital anesthesia on GD 21(08.00 h), and their fetuses were collected through uterectomy (Singh and Tripathi, 2015; Singh et al., 2016a). The remaining half of the dams of each group ($n = 6$/group) survived and allowed to delivered naturally.

2.4. Histopathological observations of the hippocampus of prenatally RIS exposed fetuses

Some randomly selected fetuses from each group ($n = 6$) were dissected for their brains and processed for histopathology as per standard protocol ((Singh and Tripathi, 2015; Singh et al., 2016a). In brief, the fetal brain was re-fixed in formalin, dehydrated in ascending grades of alcohol, incubated in molten paraffin wax (58 °C) and embedded in paraffin blocks, then serially sectioned at 7 μ by rotary microtome and transferred on egg albumin coated on glass slides; and finally stained with H&E or crysal violet. For analysis of hippocampus thickness, five alternate sections from each brain per dams ($n = 30$ sections/group) were selected. The relevant hippocampal layers in the coronal brain section of control and RIS exposed fetuses were identified with help of atlas (Altman and Bayer, 1995), and imaged under Eclipse CCD camera of Nikon 831 light microscope. These photomicrographs were used for measuring the thickness of the different neuronal layers of neocortex by using Image-J software (1.46r).

2.5. In situ detection of apoptosis by electron microscopy

Detailed procedure for EM observation has already been reported in our preceding publications (Singh and Tripathi, 2015; Singh et al., 2015), following the standard protocol for EM studies. In brief, ultrastructural apoptotic neurodegenerative changes in the hippocampus region of the fetal brain of control and prenatally RIS exposed rat fetuses were observed by using transmission electron microscopy. Immediately after uterectomy, fetuses were anesthetized and perfused transcardially with PBS followed by fixative (2.5% glutaraldehyde and 2% paraformaldehyde, in 0.1 M phosphate buffer (pH 7.4) for 6–12 h. The brains were quickly removed from the cranium, placed on ice and hippocampal region were dissected out and hippocampal tissues were cut into 1 × 1 mm size. Small pieces of 1 mm size were fixed overnight in the same fixative and processed as per standard protocol and embedded in epon resin blocks. Ultrathin sections (50 nm) were cut on Ultramicrotome