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Paliperidone reverts Toll-like receptor 3 signaling pathway activation and cognitive deficits in a maternal immune activation mouse model of schizophrenia

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ABSTRACT

The pathophysiology of psychotic disorders is multifactorial, including alterations in the immune system caused by exogenous or endogenous factors. Epidemiological and experimental studies indicate that infections during the gestational period represent a risk factor to develop schizophrenia (SZ) along lifetime. Here, we tested the hypothesis that the antipsychotic paliperidone regulates immune-related brain effects in an experimental model of SZ. A well described prenatal immune activation model of SZ in mice by maternal injection of the viral mimetic poly(I:C) during pregnancy was used. Young-adult offspring animals (60PND) received paliperidone ip (0.05 mg/kg) for 21 consecutive days. One day after last injection, animals were submitted to a cognitive test and brain frontal cortex (FC) samples were obtained for biochemical determinations. The adults showed an activated innate immune receptor TLR-3 signaling pathway, oxidative/nitrosative stress and accumulation of pro-inflammatory mediators such as nuclear transcription factors (i.e., NFκB) and inducible enzymes (i.e., iNOS) in FC. Chronic paliperidone blocked this neuroinflammatory response possibly by the synergic activation and preservation of endogenous antioxidant/anti-inflammatory mechanisms such as NRF2 and PPARγ pathways, respectively. Paliperidone administration also stimulated the alternative polarization of microglia to the M2 anti-inflammatory profile. In addition, paliperidone treatment improved spatial working memory deficits of this SZ-like animal model. In conclusion, chronic administration of paliperidone to young-adult mice prenatally exposed to maternal immune (MIA) challenge elicits a general preventive anti-inflammatory/antioxidant effect at both intracellular and cellular polarization (M1/M2) level in FC, as well as ameliorates specific cognitive deficits.

1. Introduction

Alterations in the innate immune system, caused by endogenous or exogenous factors, have been proposed as a key component in the pathophysiology of several psychotic diseases including schizophrenia (SZ) (revised in Leza et al., 2015). Epidemiological studies indicate that infections during the gestational period represent a risk factor for developing SZ over the course of a lifetime (Brown and Derkits, 2010). Although still not conclusive, several original studies and meta-analyses suggest that infections could facilitate the occurrence of damage in neurodevelopment, neurotransmission and sensorial information processing, which may play a role in the emergence of SZ (Arias et al., 2012; Khandaker et al., 2015).

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Considering this evidence, animal models based on maternal immune activation (MIA) are currently used for the study of SZ (Meyer et al., 2005; Zuckerman et al., 2003). The synthetic analogue of the viral double stranded RNA polyinosinic-polycytidilic acid (poly(I:C)) is a Toll-like Receptor 3 (TLR3) agonist that, administered to pregnant mice, elicits immune responses analogous to those observed in viral infections (Meyer, 2014). Stimulation of the innate immune system through TLR3 triggers several intracellular signaling pathways that induce antiviral and inflammatory responses mediated by the transcription factors interferon regulatory transcription factor 3 (IRF3) and the nuclear factor κB (NFκB), respectively (Matsumoto and Seya, 2008). This acute inflammatory response is mainly characterized by the secretion of pro-inflammatory cytokines (Cunningham et al., 2007). The imbalance of cytokine milieu in accordance with the excessive production of reactive Oxygen and Nitrogen species and pro-inflammatory mediators could compromise the normal course of fetal neurodevelopment and predispose to long-lasting structural/functional brain abnormalities, leading to the emergence of psychopathological behavior in adulthood (Meyer et al., 2009; Venkatasubramanian and Debnath, 2013). TLR3 activation during gestation inhibits cortical neurogenesis, synaptic transmission and behavioral abnormalities in the rodent offspring (De Miranda et al., 2010; Zuckerman et al., 2003). All these changes contribute to yield a neurodevelopmental altered animal model that displays face, construct and predictive validity for schizophrenia studies (Ozawa et al., 2006).

In response to immune activation, not only pro-inflammatory, but also anti-inflammatory and antioxidant mechanisms are activated in the central nervous system (CNS). The antioxidant pathway regulated by the nuclear transcription factor (erythroid-derived 2)-like 2, NRF2 is the first example of the stated antioxidant mechanism. Under basal conditions, NRF2 is inactivated in the cytoplasm by binding to Kelch-like ECH-associated protein 1 (KEAP1). In presence of oxidative stress signals, NRF2 translocates into the nucleus where it binds to consensus sequences of antioxidants response elements (ARE). ARE encode a wide variety of antioxidant enzymes including some dedicated to glutathione synthesis and to the elimination of oxygen reactive species (Zhang et al., 2013). On the other hand, activation of the *gamma* isoform of peroxisome proliferator-activated nuclear receptors (PPARγ) by endogenous/synthetic ligands produces a multifaceted anti-inflammatory/antioxidant and an anti-excitotoxic and pro-energetic response in different CNS pathologies (García-Bueno et al., 2008). Moreover, the polarization of microglia to the anti-inflammatory phenotype M2 secretes anti-inflammatory cytokines such as the transforming growth factor *beta* (TGFβ) and interleukin 10 (IL10) (Hu et al., 2015). Increased M2 profile could provide neuroprotection under CNS pathological conditions, and its modulation is emerging as a promising target in neuropsychiatric diseases (Reus et al., 2015).

The long-term mechanisms of action of antipsychotic drugs remain unclear since their therapeutic effects are not fully explained by their action on dopaminergic and serotonin receptors. Previous studies showed that several atypical antipsychotics, including risperidone, present an anti-cytokine effect in *in vivo*/*in vitro* settings (Kato et al., 2007; MacDowell et al., 2013; Sugino et al., 2009). Some effects of antipsychotics on anti-inflammatory/oxidant pathways have also been described (Drzyzga et al., 2006; MacDowell et al., 2016) but their potential direct actions on innate immunity remain unexplored. This study aims to elucidate the effects of paliperidone on MIA-induced cognitive dysfunctions and activation of TLR-3, neuroinflammatory and counterbalancing NRF2 and PPARγ pathways.

2. Material and methods

2.1. Animals and experimental model

Pregnant C57BL/6J mice (Harlan Ibérica, Spain) were injected i.p. with either 5 mg/kg poly(I:C) (Sigma-Aldrich, Spain) or the vehicle (saline solution) on gestational day 9.5 (Holloway et al., 2013). Animals were maintained under standard temperature and humidity conditions in a 12 h light/dark cycle (lights on at 08:00) with free access to food and water. Experimental protocols were approved by the Animal Welfare Committee of the University of Basque Country in accordance with European legislation (D2010/63/UE). This prenatal immune activation induces behavioral and brain structural/functional anomalies in adult offspring (Meyer, 2014). See Fig. S1 in the supplementary information (SI) for details.

2.2. Drug administration and experimental designs

Male and female pups were born from three poly (I:C)-treated and four saline-treated dams and were randomly assigned among four treatment groups with variables of pre-treatment (poly(I:C) vs. saline) and drug (paliperidone vs. vehicle). The atypical antipsychotic paliperidone (3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one) (PubChem CID: 115237, Sigma-Aldrich, Spain) was dissolved in a saline solution with 0.26 mM of acetic acid (vehicle, Veh), pH adjusted to 7.4 with NaOH. Young-adult animals (≥60 postnatal days; PND) originated from the seven litter were injected i.p. with either paliperidone (P; 0.05 mg/kg) or the vehicle for 21 consecutive days. Group sample sizes were poly(I:C)/Veh, n = 9 (4 female and 5 male); poly(I:C)/P, n = 8 (4 female and 4 male); saline/Veh, n = 11 (5 female and 6 male); saline/P, n = 8 (3 female and 5 male) (see Fig. S1 in SI). The dose of paliperidone was selected to be similar to commonly prescribed human dosages for a 50 kg adolescent, and on the basis of previous *in vivo* determinations of behavioral and brain structural abnormalities in adulthood in poly(I:C) offspring (Piontkewitz et al., 2011; Richtand et al., 2011). No differences in body weight between the four animal groups were observed.

2.3. Behavioral test

Animals were submitted to an alternation task T-maze test (Holloway et al., 2013). The full experiment consisted of three parts: habituation, training, and testing. During the three periods, animals were partially food-deprived. Habituation and training were performed whereas animals were under treatment with paliperidone or vehicle. For habituation, animals were placed on the T-maze with food spread. This was repeated three times a day for 5 days. During training, animals received six trials a day. Each training trial consisted of two runs, a forced and a free run. In the forced run, mice were forced to obtain a piece of food from goal alley of the T-maze, with the other alley blocked by its door. Then, animals were placed back into the start arm for 10 and 40 s delay periods (three trials for each delay period). At the free run, animals were allowed to choose either goal alley. If the mice chose the same arm into which they had been forced, they did not receive food reward whereas if they chose the opposite arm they received food reward and the choice was considered correct. The sequence of delays and forced-run food locations were randomized each day. Animals received six trials a day with a 5 min intertrial interval. It was considered that animals were trained when controls mice made more than 70% correct choices on two consecutive days. Animals reaching this threshold were maintained under daily training until chronic paliperidone/vehicle treatment was finished (21 days). The test day (24 h after