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Impacts of early intervention with fluoxetine following early neonatal immune activation on depression-like behaviors and body weight in mice

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ABSTRACT

Several reports have suggested that early neonatal immune activation adversely influences the hypothalamic–pituitary–adrenal (HPA) axis development in humans and animal models. In addition, there have been several studies indicating that early intervention with fluoxetine (FLX) can alter HPA axis development and function, and prevent occurrence of behavioral abnormalities induced by common early-life insults. The present study aims to investigate the effects of early intervention with FLX following early neonatal immune activation on depression-like behaviors and body weight in mice. Neonatal mice in their postnatal days (PNDs) 3 and 5 received either lipopolysaccharide (LPS; 50 μg/kg, s.c.) or saline treatment, then male and female mice of both neonatal intervention groups received oral administration of FLX (5 and 10 mg/kg/day) or water via regular drinking bottles during the periadolescent period (PNDs 35–65). The results showed that neonatal LPS exposure elevated depression-like behaviors accompanied by increasing corticosterone levels in adulthood and decreasing body weight during neonatal and adolescent periods. Furthermore, the periadolescent FLX treatment inhibited the depression-like behaviors induced by neonatal infection in both sexes. This study obtained some experimental evidence indicating the potential adverse impacts of the FLX on normal behavioral development in male control animals. In conclusion, our findings suggest that an early pharmacological intervention with FLX may prevent emergence of depression-like behaviors induced by neonatal immune challenge without any detrimental effect on health in a sex- and dose-dependent manner in mice.

1. Introduction

There is a great deal of evidence from human and animal studies indicating that adverse events in neonatal period can negatively affect the trajectory of normal brain development and function of physiological and behavioral systems across the life span (Korosi et al., 2011; Lee and Dammann, 2011; Pesonen and Räikkönen, 2011; Skripuletz et al., 2010; Walker et al., 2011; Zakharova, 2009). Several experimental models have proven a significant link between neonatal exposure to inflammatory agents like lipopolysaccharide (LPS) and increased likelihood of neuropsychiatric disorders in later life. In this context, multilaboratory studies have shown that LPS-induced neonatal immune activation alters hypothalamic–pituitary–adrenal (HPA) axis activity (Nilsson et al., 2002; Shanks et al., 1995, 2000) resulting in modifications of physiological (Iwasa et al., 2010), immunological (Boissé et al., 2004), behavioral (Shanks et al., 1995), and neuroendocrine (Iwasa et al., 2009) systems in adulthood.

Previous studies have indicated that LPS exposure on postnatal days (PNDs) 3 and 5 facilitates anxiety-like behaviors in adult rats (Sominsky et al., 2011; Walker et al., 2004, 2009). However, little attention has so far been devoted to the evaluation of early postnatal inflammation impacts on depression-like behaviors in animal models. In this regard, we and others have shown that there might be an association between anxiety and depression-like behaviors (Beuke et al., 2003; Enayati et al., 2012) in which genes likely have a crucial role as well as the etiology of these two behaviors (Field et al., 2010; Kendler et al., 2007; Williamson et al., 2005). Although,

Abbreviations: LPS, lipopolysaccharide; HPA, hypothalamic–pituitary–adrenal; PND, postnatal day; FLX, fluoxetine; SSRI, selective serotonin reuptake inhibitor; COR, corticosterone; FST, forced swimming test; TST, tail suspension test; ANOVA, analysis of variance; NPY, neuropeptide Y; IL, interleukin; TNF-α, tumor necrosis factor-alpha; BDNF, brain derived neurotrophic factor.

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previous studies have shown the importance of evaluating sex impacts on adverse outcomes of prenatal and neonatal immune activation (Boksa, 2010; Darnall and Suarez, 2009; de Vries and Södersten, 2009; Harvey and Boksa, 2012; Rana et al., 2012), the majority of the studies have exclusively been conducted on the male subjects. This is a problematical issue given that many neuroscientists have indicated a broad range of sex differences in main characteristics of neuropsychiatric disorders (Ngun et al., 2011). Therefore, it is an interesting research question whether early postnatal immune challenge can result in depression-like behaviors in adult male and female mice.

Several researchers have attempted to develop therapeutic strategies for treating or preventing neuropsychiatric disorders with neurodevelopmental origins like schizophrenia and depression. These studies have demonstrated that early intervention with antipsychotic and antidepressant drugs, including clozapine, haloperidol and fluoxetine (FLX) effectively reduces behavioral disturbances and structural abnormalities in different brain regions, induced by maternal immune activation, prenatal stress and neonatal maternal separation in offspring. Nevertheless, some adverse effects of these drugs have also been reported in control animals (Dickerson et al., 2012; El Khoury et al., 2006; Ishiwata et al., 2005; Lee et al., 2001; Meyer et al., 2010; Nagano et al., 2012; Pawluski et al., 2012; Piontkewitz et al., 2009, 2011; Rayen et al., 2011; Richtand et al., 2012; Yoo et al., 2012).

FLX is a selective serotonin reuptake inhibitor (SSRI) used for the treatment of psychiatric disorders especially depression, in humans (Walker, 2012). It is well documented that FLX affects serotonergic system and serotonin plays a pivotal role in brain development (Gaspar et al., 2003; Whitaker-Azmitia et al., 1996) through its role in the regulation of proliferation, differentiation, survival of newborn neurons, neurogenesis, synaptogenesis, and dendritic growth (David et al., 2009; Fujioka et al., 2004; Malberg et al., 2000; Walker, 2012). Interestingly, previous studies have shown paradoxical responses of mice to SSRIs during a specific postnatal period (PNDs 4–21) of development through both decreased exploratory behavior and increased anxiety- and depression-like behaviors in adulthood (for review, see Oberlander, 2012). In this regard, a meta-analysis study showed that some SSRIs, including FLX, sertraline, and citalopram had more beneficial effects to treat depression in children and adolescents, compared with other SSRIs (Usala et al., 2008). In addition, Ishiwata et al. demonstrated that FLX treatment during postnatal weeks 1–3 in the mice, exposed to prenatal stress, normalizes corticosterone (COR) responses to a subsequent stressor, compared with the impacts of exposure to prenatal stress alone (Ishiwata et al., 2005). Recently, a similar study has shown that adolescent FLX treatment improves depression-like behaviors in female rats that experienced neonatal maternal separation (Yoo et al., 2012). On the whole, these evidences show that postnatal FLX treatment may have neutral, beneficial, or even detrimental impacts on early neurodevelopment.

Therefore, the present study aims to investigate the effects of early intervention with FLX following early neonatal immune activation on the development of depression-like behaviors and body weight in male and female mice. Furthermore, to examine whether a chronic regimen of FLX can reverse behavioral abnormalities induced by early postnatal inflammation, the effects of the periadolescent FLX treatment were separately assessed in male and female mice.

2. Materials and methods

2.1. Animals

Male and female NMRI mice (70–80 days) were obtained from the animal house of Pasteur Institute (Tehran, Iran). Animals were housed in standard polycarbonate cages in a room with a 12:12 h light/dark cycle (lights on 08:00–20:00 h), controlled temperature (23 ± 1 °C) and had free access to food and water. These conditions were kept as a standard housing condition in all stages of experiments. All procedures of the study were performed in accordance with the ethical guidelines set by Research and Ethics Committee of the Tabriz University of Medical Sciences which completely coincide with the "National Institutes of Health NIH Guide for the Care and Use of Laboratory Animals (NIH; Publication No. 85-23, revised 1985).

2.2. Newborn mice

Following a 2-week period of acclimatization to the new animal housing room, to facilitate the mating, male and female mice were kept together one-by-one in a cage. Female mice were visually monitored daily for confirmation of pregnancy, when it was confirmed the female mice were removed from the breeding cages and housed individually in standard cages. All pregnant animals were allowed to have normal delivery and the first day of birth was considered PND 0. One day after the birth, all litters were culled to 10 pups per mother (5 female and 5 male). On the day 21, litters were weaned by removal of the mother and then were housed with the same sex litter-mates (4 animals per cage). A total of thirty litters were used during this study in three stages, each of which included 10 litters. Only one mouse per dam was used for each of the experiment to avoid the litter-effect.

2.3. Neonatal immune activation

A timeline diagram of the experiments is shown in Fig. 1. Pups from 30 litters were divided into 2 clusters, each of them consisted of twelve main groups (8 male and 8 female mice in each group): Cluster 1 ($n = 96$) included saline injected mice and Cluster 2 ($n = 96$) LPS injected mice. The dams were removed from their pups for approximately 10 min and the pups were weighed and received subcutaneously (in the interscapular region) injection of LPS (*Escherichia coli* 0111:B4, Sigma Co, USA; 50 μg/kg) or vehicle solution (1 ml/kg) on PNDs 3 and 5. These PNDs correspond to the third trimester of human pregnancy when major brain growth occurs. The dose and timing of LPS treatment were chosen based on the previous studies (Breivik et al., 2002; Knox et al., 2009; Li et al., 2007; Rico et al., 2010; Sominsky et al., 2011; Tenk et al., 2007, 2008; Walker et al., 2004, 2006a, 2008, 2009, 2010, 2011; Wu et al., 2011). The LPS was dissolved in sterile saline (0.9% NaCl) and injections were performed between 12:00 and 13:00 P.M. Each injection was performed through a 27-gauge needle connected by polyethylene tubing to a 10-μl Hamilton syringe. Newborn mice were returned to their housing immediately following saline or LPS injection.

2.4. Drug treatment during periadolescent

The periadolescent FLX hydrochloride (0060108; TEMAD Co., Tehran, Iran) treatment (5 and 10 mg/kg/day) was initiated on the PND 35 and lasted for 30 days (PND 65; see Fig. 1) (Meyer et al., 2010). The FLX doses were chosen based on previously published protocol specified to an oral drug administration via regular drinking bottles in mice (Dulawa et al., 2004). The FLX was dissolved in the drinking water and its concentration was calculated at four-day intervals according to the average liquid consumption and body weight per cage in our laboratory. The mice did not receive any source of water except for the drinking water containing FLX solutions. Therefore, they were motivated by thirst to drink the drug solutions. To examine possible effects of the chronic FLX treatment, the liquid consumption of the control mice was measured every 4 days which showed no significant change in the liquid intake compared with the drug-receiving mice (as a consequence of the chronic antidepressant drug exposure during adolescence) (Meyer et al., 2010).