Neurotoxicology and Teratology 56 (2016) 1–8

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Full length article

Co-exposure to fish oil or folic acid does not reverse effects in the progeny induced by maternal exposure to fluoxetine

Andressa Keiko Matsumoto [a], Carolina Matias Higashi [a], Kamila Landucci Bonifácio [b], Matheus Azevedo Barbosa [a], Rodrigo Moreno Klein [c], Guilherme Bracarense Filgueiras [a,c], Graziela Scalianti Ceravolo [a], Estefânia Gastaldelo Moreira [a,b,*]

[a] Department of Physiological Sciences, State University of Londrina, Londrina, PR, Brazil
[b] Graduation Program in Health Sciences, State University of Londrina, Londrina, PR, Brazil
[c] Department of Psychology and Behavioral Analysis, State University of Londrina, PR, Brazil

ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form 13 May 2016
Accepted 19 May 2016
Available online 20 May 2016

Keywords:
Fluoxetine
Antidepressants
Folic acid
Fish oil
Brain development

ABSTRACT

The serotonin reuptake inhibitor antidepressant fluoxetine (FLX) has been widely prescribed for the treatment of some diseases during pregnancy and/or lactation. Despite possible adverse effects observed in the progeny after maternal exposure to FLX, it is recognized that not treating a mother who has clinical indication for antidepressants may also impact negatively her well being and the fetal/newborn development. The present study investigated if two supplements already used by pregnant women, i.e., folic acid (FA) and fish oil (FO) could minimize effects in pubertal rats induced by maternal exposure to FLX during pregnancy and lactation. Evaluations included two behavioral tests (elevated plus maze and novelty-induced suppressed feeding) and quantification of brain derived neurotrophic factor (BDNF) in the hippocampus. Wistar rats were gavaged with FLX (5 mg/kg) or tap water associated or not with FA (3 mg/kg) or FO (1.3 mg/kg) during pregnancy and lactation. Pups exposed to FLX presented anxiety-like behavior and decreased hippocampal BDNF levels that were not influenced by co-exposure with FA or FO. FO exposure resulted in anxiety-like behavior, decreased BDNF levels and eating behavior whereas FA exposure increased BDNF and induced hyperactivity in the elevated plus maze. In conclusion, this study suggests that reduced hippocampal BDNF may play a role in the developmental neurotoxicity of FLX. FA and FO were not effective as employed in the present study but the search for alternatives to manage the risk of antidepressant exposure during pregnancy and/or lactation is likely to continue.

1. Introduction

Antidepressants are extensively used worldwide. They are prescribed not only for the treatment of depression and dysthymia but also for the treatment of anxiety disorders and prophylaxis of pain syndromes (e.g., fibromyalgia, migraine). Since the therapeutic effects of antidepressants result from neuroadaptations, treatment with these drugs is long lasting. Women who use antidepressants and get pregnant may not have the indication to interrupt the treatment under the risk of relapse or recurrence of the disease. Moreover, pregnant and nursing women may also need to use antidepressants if they develop a psychiatric illness during these periods.

The selective serotonin reuptake inhibitor (SSRI) antidepressants are the most used antidepressants during pregnancy (Forsberg et al., 2014; Jimenez-Solem et al., 2013; Kassada et al., 2015; Zoega et al., 2015). The specific SSRI used for pregnant women varies according to the country and period of data collection but fluoxetine (FLX) is among the most used ones (Forsberg et al., 2014; Jimenez-Solem et al., 2013; Kassada et al., 2015; Zoega et al., 2015).

FLX is known to cross the placental barrier in humans and rodents (Hendrick, 2003; Pohland et al., 1989) and to be excreted in milk (Heikkinen et al., 2003; Hendrick et al., 2001; Suri et al., 2002). In this way, maternal use results in fetus and neonates exposed to increased levels of serotonin at critical stages of brain development and it is known that 5-HT acts as a neurotrophin and influences migration, axon growth, synaptogenesis, circuit wiring and neurogenesis (Homberg et al., 2010; Lauder, 1990; Whitaker-Azmitia et al., 1995).

Behavioral and non-behavioral effects have been reported in animals after in utero exposure to SSRIs (Cabrera-Vera et al., 1997; Cabrera-Vera and Battaglia, 1998; Noorlander et al., 2008; Olivier et al., 2011; Smit-Rigter et al., 2012). However, studies reporting lack of effects have also been published (Bairy et al., 2007; Capello et al., 2011). Different results observed in the rodents` progeny by different laboratories after developmental exposure to FLX or others SSRIs

* Corresponding author at: Departamento de Ciências Fisiológicas, CCB, UEL, Lab.06, CEP 86051-980 Londrina, PR, Brazil.
E-mail address: egmoreira@uel.br (E.G. Moreira).

http://dx.doi.org/10.1016/j.ntt.2016.05.006
0892-0362/

would be expected since the designs of the studies differ on issues widely known to influence developmental neurotoxicity endpoints, such as time of exposure, dose, age of evaluation, etc. However, disrupted emotional behaviors in the progeny after maternal exposure to FLX are commonly reported (Lisboa et al., 2007; McAllister et al., 2012; Noorlander et al., 2008; Olivier et al., 2011; Smit-Rigter et al., 2012).

Anxiety and depressive-like behaviors have been linked to decreased hippocampal neurogenesis. The neurotrophin brain derived neurotrophic factor (BDNF) is a key player in neurogenesis and neuronal plasticity and the therapeutic effects of antidepressants that increase 5-HT and/or noradrenaline availability are partially mediated by an increase in BDNF levels (Nestler et al., 2008).

Our group has been investigating the potential developmental neurotoxicity of FLX using the principles published in the guideline 426 from the Organization for Economic Co-Operation and Development (OECD 2007). So far, we have reported that exposure during gestation and lactation to doses of FLX that do not influence maternal and litter weight: decreased ambulation of pubertal male mice in an open-field (Lisboa et al., 2007); increased depressive-like behavior of pubertal and adult female mice (Lisboa et al., 2007); decreased behavioral response to dopaminergic drugs of pubertal female mice (Favaro et al., 2008); decreased sexual motivation of adult mice (Gouvêa et al., 2008); altered decision-making behavior of male and female pubertal rats (Francis-Oliveira et al., 2013); decreased stress-induced activation of the basolateral amygdala of pubertal and adult male rats (Francis-Oliveira et al., 2013).

Despite possible adverse effects observed in the progeny after maternal exposure to FLX, it is recognized that not treating a mother who has clinical indication for antidepressants may also impact negatively her well being and the fetal/newborn development.

Little is known about the molecular changes induced by exposure to FLX during brain development but our group recently reported that this exposure decreases global DNA methylation profile in the hippocampus of weaned rats (PND 22) (Toffoli et al., 2014). Since DNA methylation is known to play an important role in epigenetic reprogramming (i.e., the control of gene expression in early stages of embryonic development and cell differentiation) the observed decreased methylation profile suggests that this may be one of the molecular mechanisms by which early exposure to FLX disturbs brain development and triggers mood/affective alterations later in life. Toffoli et al. (2014) also reported that co-administration of FA (8 mg/kg, gavage) reversed the FLX-induced decrease of global methylation profile. Folate is a co-factor in the biosynthesis of *S*-adenosylmethionine, the main donor of methyl group for DNA methylation, and this is the mechanism by which it modifies gene expression (Cho et al., 2013; Taylor et al., 2004).

Based on the above considerations, we designed this study in order to evaluate if two supplements already used by pregnant women, i.e., folic acid (FA) or fish oil (FO) could minimize the effects induced by maternal exposure to FLX in the exposure model that has been employed in our laboratory. We decided to use only pubertal rats (35 days) considering that our previous studies (listed above) indicated this period as a sensitive one. Evaluations included two behavioral tests (elevated plus maze and novelty-induced suppressed feeding) and quantification of the hippocampal neurotrophin brain derived neurotrophic factor (BDNF).

2. Material and methods

2.1. Animals and treatment

Male and female Wistar naive rats were mated overnight (1 male and 3 females per cage), and gestational day 0 (GD 0) was determined through vaginal smear, by the presence of spermatozoids and estrous phase. Each female was housed individually in controlled environmental conditions, i.e., temperature at 21 ± 2 °C, 12 h dark/light cycle (lights on at 06:00 AM) and free access to food and tap water.

Dams were allocated into 2 groups and gavaged daily with tap water (H_2O) or 5 mg/kg FLX (Daforin® oral solution, EMS, Brazil). Each one of these groups were subdivided into 3 groups that received, 1 h after the first gavage, H_2O, 3 mg/kg folic acid (FA, oral solution, Fagron, Brazil) or 1.3 g/kg fish oil (FO, Herbarium Botanical, Brazil) containing 12% eicosapentaenoic acid and 18% of docosahexaenoic acid, i.e., 400 mg/kg omega-3. In this way, there were 6 groups in total: H_2O-H_2O (13 l); H_2O-FA (15 l); H_2O-FO (14 l); FLX-H_2O (15 l); FLX-FA (15 l); FLX-FO (13 l). The treatment lasted throughout the pregnancy and lactation.

At birth, i.e., post-natal day (PND) 0, the liters were weighed and the number of live and dead pups counted. At PND 4, liters were culled to 10 pups and liters smaller than 8 pups were discarded. The liters were weighed on PND 0, 7, 14 and 21, when they were weaned and housed separated by sex until their evaluation, which occurred on PND 35. From each litter, only one male and one female pup were used for each evaluation, i.e., the litter was the experimental unit.

Dams were weighed daily in order to adjust dosing. The rationale for FLX dosing is described in Lisboa et al. (2007) but in the present study we calculated for rats and not mice. The dose of FA recommended for pregnant women with risk of fetal developmental problems related to neural tube is 5 mg/day or 0.071 mg/kg for a 70 Kg woman. In order to account for inter-species variation, we applied dosimetry $BW^{3/4}$ (EPA 2006) and obtained 0.3 mg/kg and then used a 10 factor to account for intra-species variation. Therefore, the selected FA dose for this study was 3 mg/kg/day. For FO a similar rationale was applied considering the recommended omega-3 dose for humans of 900 mg/day or 1.3 mg/kg.

All the experimental protocols were approved by the State University of Londrina Ethics Committee for Animal Research (protocol 3058.2014.58).

2.2. Behavioral analysis

The pups from both sexes were used only once. Behavioral tests were recorded by a video camera, linked to a computer in an adjacent room. Videos were analyzed blindly to treatment.

2.3. Novelty-supressed feeding (NSF)

This test was adapted from Bodnoff et al. (Bodnoff et al., 1989) and is considered to evaluate anxiety and depression-related symptoms (Bodnoff et al., 1989; Dulawa and Hen, 2005). Animals were food-deprived in metabolic cages for 24 h prior to the test. Then, the rat was placed in the periphery of an arena (72 cm in diameter) containing 30 g of rodent chow in its center and the latency to begin eating was recorded. Trials lasted 5 min. Considering that treatment-induced alterations in eating behavior could bias the results from this test, the normal eating behavior of the animals was evaluated by returning them to the metabolic cage and quantifying the amount of food consumed during 5 min. The arena was cleaned with alcohol 5% between trials.

2.4. Elevated plus maze

The apparatus consisted of two open arms, 50 × 12 cm (length × width) and two closed arms, 50 × 12 × 50 cm (length × width × height) with an open roof arranged such that the two arms of each type were opposite to each other. For this test, that evaluates anxiety-related behaviors (Pellow and File, 1986), each animal was placed in the center of the maze, facing one of the open arms and the number of entries into, and the time spent in the open and the closed arms were registered for 5 min. Moreover, stretched attend posture (SAP: when the rat stretched its body without moving its hind