Effects of maternal exposure to antidepressant fluoxetine on humoral immunity in rat pups

Eduardo Vignoto Fernandes¹, Aline Camargo Ramos², Alice Hartmann dos Santos², Daniela Cristina Cecatto Gerardin² & Emerson José Venancio¹.*

¹Department of Pathological Sciences; ²Department of Physiological Sciences, State University of Londrina - UEL, Londrina, Paraná, Brazil.

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Fluoxetine is a common drug for the treatment of depression. However, their effects on the development of the offspring are still poorly understood, especially in relation to the humoral immune response. In this study, we evaluated the antibody production of rat pups from female rats administered with fluoxetine during pregnancy and lactation. Pups of both control and fluoxetine groups were first weaned and after divided into 4 subgroups: male control; female control; male fluoxetine and female fluoxetine. The animals were administered subcutaneously with 50 μL of PBS solution containing 50 μg of chicken immunoglobulin Y (IgY) and 500 μg aluminium hydroxide on post-natal day 26 and 40. Blood collection was performed 7 days after each administration. The titres of IgM, IgG1 and IgG2a antibodies were determined by ELISA. Results were submitted to analysis of variance and the differences between averages were compared by Bonferroni post hoc test, P <0.05. The results showed increased production of IgG1 and IgG2a (anti-IgY) antibodies in animals exposed to fluoxetine. In relation to gender, an increase in the production of antibodies (IgG1) was seen only in females. The data obtained indicates that maternal exposure to fluoxetine can modulate the antibodies production in offspring.

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groups: control group (CON): 10 dams received 0.25 mL of tap water daily (by oral gavage) from GD 0 to post-natal day (PND) 21; and fluoxetine group (FLX): 10 dams received 7.5 mg/kg of FLX (Daforin® liquid, Novaquimica, Brazil) daily by oral gavage from GD 0 to PND 21. The dose of FLX was based on the weight of the dams on GD 0 and it was maintained until the end of the lactation period. On PND 21, the pups were weaned and divided into four subgroups: male control, MCON; female control, FCON; male exposed to fluoxetine, MFLX; and female exposed to fluoxetine, FFLX. This experiment was done only once.

All the experimental protocol was approved by the State University of Londrina Ethics Committee for Animal Research (protocol number: ECAE 102/09).

Immunization and blood collection

On PND 26 and 40 of life, all animals were administrated subcutaneously with 50 µL of a solution containing 50 µg of chicken IgY antibody and 500 µg aluminium hydroxide diluted in phosphate buffered saline (PBS), pH 7.4. On PND 26 (Pre, before immunization), 33 (Post1, seven days after the first immunization) and 47 (Post2, 7 days after the second immunization), all animals were sedated by non-lethal inhalation of ethyl ether and approximately 0.5 mL of blood was collected by cardiac puncture. The collected blood was stored in 1.5 mL plastic tubes containing 50 μL of 5% EDTA.

Enzyme-Linked Immunosorbent Assay (ELISA)

To assess the anti-chicken IgY antibodies production (IgM, IgG1 and IgG2a), an enzyme-linked immunosorbent assay (ELISA) was carried out. To each well, 100 µL of a solution of 1 µg/mL chicken IgY antibody was added to sensitise the plate. The plasma was diluted 1:100. The dilutions of peroxidase conjugated anti-rat IgM, anti-rat IgG2a (manufactured by Zymed) and anti-rat IgG1 (produced by BETHYL) were 1:5 000, 1:5 000 and 1:50 000, respectively. The ELISA was conducted as described by Fernandes et al.\textsuperscript{11}.

Statistical analysis

Initially, an exploratory analysis was conducted to evaluate the normal distribution (Kolmogorov-Smirnov test) and the homogeneity of variance (Levene’s test) of each variable. After verifying that the distribution of the data was normal and homogeneous, parametrical analyses were conducted. To evaluate the effect of fluoxetine on antibody production (IgM, IgG1 and IgG2a) and to verify whether there were differences between groups (MCON, MFLX, FCON and FFLX) or not, the one way ANOVA test was applied. To verify whether there were differences throughout the time (Pre, Post1 and Post2), the repeated measures ANOVA was realized. Whenever necessary the Bonferroni post hoc test was conducted. The differences were considered significant at $P <0.05$.

Results

Effects of fluoxetine on the production of antibodies

Fig. 1 shows the levels of specific antibodies (IgM, IgG1 and IgG2a), anti-chicken IgY, before immunization (Pre, 26 PND), 7 days after the first immunization (Post1, 33 PND) and 7 days after the second immunization (Post2, 47 PND). In relation to the immunization, all the pups increased its levels of specific IgM, IgG1 and IgG2a antibodies. However, in the 47 PND only IgG1 and IgG2a antibody class showed a higher in level, compared to 33 PND. Furthermore, maternal exposure to fluoxetine resulted in an increased on the level of anti-IgY IgG1 and IgG2a antibodies in pups (Fig. 1 B and C, $P <0.05$).

On the other hand, no significant difference was observed on the levels of IgM produced by offspring treated and non-treated with fluoxetine.

Interaction between drug and gender

A significant difference on the levels of specific IgG1 antibodies was observed in female offspring of dams treated with fluoxetine, compared with female offspring of dams not treated with fluoxetine (Fig. 2B, $P <0.05$). No significant differences were observed in relation to IgM and IgG2a (anti-IgY) antibodies (Fig. 2 A and C, $P >0.05$). As expected, all the animals increased their levels of IgM, IgG1 and IgG2a (anti-IgY) subclasses had higher antibodies levels in relation to 33 PND (after the second immunization).

Discussion

This study investigated the effects of fluoxetine exposure during pregnancy and lactation on the humoral immune response of male and female rat pups. Various studies have investigated the immunomodulatory effects of fluoxetine. In animal models, it was observed that the treatment with fluoxetine resulted in increased of peripheral blood TCD8+ cells and decreased peripheral blood TCD4+.
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It is probable that the immunomodulatory action of fluoxetine is associated with cytokine production. In murine cancer models, treatment with fluoxetine (15 mg/kg) results in the increased production of pro-inflammatory cytokines (IFN-γ and TNF-α), a lower rate of tumour growth and a longer survival time. In mice, stimulated with LPS, pre-treatment with fluoxetine (5, 10, 15 and 20 mg/kg) leads to a reduction of up to 60% on the levels of TNF-α and to a 50% of mortality. A similar anti-inflammatory
The influence of the nervous system on the immune system seems to be dependent on the sex of the animal. A study with male and female rats stimulated with LPS early after birth showed that female rats have higher plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone in relation to males. Interestingly, immune activation with LPS in neonatal mice resulted in an increase in corticosterone and depressive behavior in both genders, which were reversed by the treatment with fluoxetine. Furthermore, there is evidence that corticosterone can modulate the level of specific antibody production in mice. Thus, knowing that corticosterone has immunosuppressive action when its plasma levels persists elevated and that females produce more cortisol when immunologically challenged, the results obtained in this work showed that the treatment with fluoxetine inhibited the stressful effects of the immunization, favouring the antibodies production.

In conclusion, the present study showed that the treatment of female Wistar rats with fluoxetine during pregnancy and the lactation period could lead to regulation of HPA axis and in the normal production of the antigen-specific antibody in the offspring. This effect was mainly found due to the action of fluoxetine on the females. However, new studies, which could investigate the possible mechanisms that lead females to produce fewer antibodies when submitted to immune stress and what would be the real role of fluoxetine in this context, are required.

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Conflict of interest statement
We declare that we have no conflict of interest.

References