Acta Histochemica 117 (2015) 582–589

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Effects of treatment with fluoxetine on mandibular development: A morphological study in rats

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ARTICLE INFO

Article history:
Received 11 January 2015
Received in revised form 28 May 2015
Accepted 29 May 2015

Keywords:
Antidepressants
Bone
Fluoxetine
Mandible
Serotonin

ABSTRACT

Aim: To verify whether the use of fluoxetine during gestation and lactation interferes in mandibular bone formation in rats.
Methods: Twenty-four *Wistar* rat pups were used and distributed into four groups: CG – control of gestation; CL – control of gestation and lactation; FG – treated with fluoxetine during gestation and FL – treated with fluoxetine during gestation and lactation. At 25 days of life, after anesthesia, perfusion and decapitation, the mandibles were removed. Radiographic, histologic, histometric and polarizing microscopy analyses were performed. Statistical analysis was used considering a level of 5% significance.
Result: The FL group compared with its control (CL) was shown to differ statistically from the other groups as regards histometry and radiopacity, revealing a reduction in the inferior cortical thickness, reduction in number of osteocytes, with consequent reduction in radiographic bone density. There was also reduction in the number of osteoblasts in FG.
Conclusion: The long-term use of fluoxetine via oral route by pregnant and lactating rats modifies the mandibular bone mass.

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1. Introduction

Depression is a health problem with worldwide repercussions and is frequently observed during pregnancy, with up to 14.5% of pregnant women affected by the symptoms of depression (Sit et al., 2011) and 10–15% in the post-partum period (Pawluski et al., 2012). When depression develops during pregnancy and persists until the post-partum period, it has negative effects on the mother, child and the entire family (Bener et al., 2012), and on the child's cognitive and emotional development (Berle and Spigset, 2011).

Depressive pregnant women, or mothers in the post-partum period may be treated with psychotherapy and/or pharmacological agents. Selective serotonin reuptake inhibitors (SSRI) are the class of drugs most used, with fluoxetine hydrochloride being widely prescribed (Jimenez-Solem et al., 2012), not only due to its efficacy, but also because of high tolerance to it (De Fátima et al., 2005). These medications selectively and potently block the serotonin transporters and receptors in the central nervous

Abbreviations: CG, control of gestation; CL, control of gestation and lactation; cm, centimetre; EDTA, ethylenediamine tetraacetic acid; ET, exposure time; FG, treated with fluoxetine during gestation; FL, treated with fluoxetine during gestation and lactation; g, gram; HE, hematoxylin and eosin; SSRI, selective serotonin reuptake inhibitors; i.m., intramuscular; JPEG, joint photographic experts group; kg, kilogram; kVp, kilovoltage; mA, milliampere; mg, milligram; mmAl, aluminum millimeter; pH, hydrogen potential; 5-HT, 5 hydroxytryptophan (Serotonina); μm, micrometer; μl, microliter.

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http://dx.doi.org/10.1016/j.acthis.2015.05.005
0065-1281/Published by Elsevier GmbH.

system, to effectively increase the extracellular levels of serotonin and alleviate the symptoms of depression (Chau et al., 2012).

The serotonin is absorbed by the cell through its transporters and binds to the cytoskeleton, reducing its motility, thereby increasing the tissue-to-tissue interaction time (Moiseiwitsch, 2000). This event marks its participation in the morphogenesis of craniofacial structures, because of the presence of 5-HT uptake sites in structures of the face (Lauder et al., 1988).

Blockade of 5-HT at the uptake site by medications during bone formation may interfere in the development of bone structures (Westbroek et al., 2007; Bonnet et al., 2007), seeing that the embryos of mice exposed to SSRI exhibited craniofacial malformations, possibly caused by the effect of this class of drug on the epithelial-mesenchymal interaction (Shuey et al., 1992).

The role of 5-HT in bone formation occurs due to the presence of its multiple receptors and transporters, which are expressed in the osteoblasts, osteocytes and osteoclasts (Bliziotes et al., 2001; Westbroek et al., 2001; Battaglino et al., 2004). These findings indicate that the bone cells have functional pathways both for responding to regulation and for 5-HT absorption (Bliziotes, 2010). This neurotransmitter participates in the growth and development of various mineralized tissues (Bliziotes et al., 2006).

Furthermore, the fundamental activity of 5-HT (serotonin) is to regulate the secretion of pituitary growth hormone (GH), which in turn, stimulates the production of insulin-like growth factors (IGFs) (Cassano et al., 2009). IGFs are essential during development, since they primarily act on bone growth, and promote differentiation of myoblasts and osteoblasts essential for development and growth (Mota et al., 1995). Therefore, a congenital GH deficiency is associated with reduced bone length at birth (Musumeci et al., 2013).

Fluoxetine may cross the placental barrier and be found in the serum of animals within the maternal uterus, at a lower serum level than that of the mother (Capello et al., 2011). Clinical reports have shown that newborns exposed to SSRI during gestation have an increased risk for developing adverse effects, such as low birth weight, lower gestational age, neuro-behavioral disturbances and reduced cardiac frequency (Oberlander et al., 2009). In addition, newborns exposed to fluoxetine have detectable plasma drug concentration levels of up to 80% (Berle and Spigset, 2011).

However, few studies have evaluated the possible alterations that may occur in the gnathic bones when exposed to the drug during formation and development. More specifically, no studies have been described with regard to the formation of basal bone of the mandible in vivo or in situ. Based on the foregoing, the aim of the present study was to verify whether the use of fluoxetine hydrochloride during gestation and lactation interferes in the bone formation of the mandibular body, reducing the size of the mandible in the offspring of rats submitted to pharmacological treatment.

2. Materials and methods

The study was approved by the Ethics Committee on Animal Research of the Federal University of Pernambuco (Protocol # 23076.017680/2011-83).

A total of 24 pups obtained from 12 pregnant rats (*Rattus novergicus, albinus, Wistar*) were housed in a vivarium where they received a standard rat diet (Presence Ratos e Camundongos, Presence, Paulinea, SP, Brazil) and water ad libitum. They were kept in a room with a temperature of $23 \pm 2\,^{\circ}C$ and light/dark cycle of 12:12 h. To obtain the newborns, adult animals were mated in the proportion of one male to three females, placed in the same cage during the night. Pregnancy was diagnosed by the vaginal smear method, and the presence of spermatozoids was detected by analysis under light microscopy, considering this as day 1 of gestation. Two pups were removed from each mother to compose the number of six pups per group: control of gestation group (CG); control of gestation and lactation group (CL); group treated with fluoxetine during gestation (FG) and group treated with fluoxetine during gestation up to the end of lactation (FL).

2.1. Pharmacological treatment

All the pregnant rats received treatment through oral gavage, once a day, applied strictly at the predetermined time (07:00 am–07:30 am), for the period of time according to each study group.

The mothers in the control groups received a 0.9% sodium chloride solution at the dose of 10 μl/g of animal weight. Group CG received the solution during gestation (from day 1 to day 20 of pregnancy) and Group CL, during the entire period of gestation and lactation (from day 1 of pregnancy to the 21st day after birth). The mothers of the treated groups received fluoxetine hydrochloride (Pharma Nostra, Rio de Janeiro, Rio de Janeiro, Brazil) at the dose of 20 mg/kg of animal weight, with Group FG being treated during gestation (from day 1 to day 20 of pregnancy) and Group FL, during the entire period of gestation and lactation (from day 1 of pregnancy to the 21st day after birth).

2.2. Obtaining the specimens

The pups were weaned at 21 days of life, and at 25 days, they were anesthetized with xylazine (Bayer Saúde Animal, São Paulo, São Paulo, Brazil) at 20 mg/kg of animal weight (i.m.) and ketamine (Cristalfarma Comércio, Representação, Importação e Exportação Ltda, Belém, Pará, Brazil) at 50 mg/kg of animal weight (i.m.). They were euthanized by perfusion via the intracardiac route, with 4% formaldehyde (ISOFAR, Duque de Caxias, Rio de Janeiro, Brazil) in a sodium phosphate (Quirios Produtos Químicos S.A, Barueri, São Paulo, Brazil) buffer (pH = 7). Afterwards, they were decapitated and their mandibles were disarticulated and sectioned in the medial portion. The right hemi-mandibles were fixed with 4% formaldehyde for 24 h at room temperature.

2.3. Evaluation of specimen radiopacity

The region analyzed was the diastema area from the right hemi-mandibular body. The radiographic exam of the specimens was performed using an intraoral X-ray appliance (Dabi Atlante, Ribeirão Preto, Brazil) operating at 70 kVp and graph 10 mA. Digora Optime® phosphorous plates (Soredex, Helsinki, Finland) were used for taking digital radiographs. The exposure time (ET) was standardized at 0.2 s. The distance used was standardized at a height of 30 cm with an acrylic focus-sensor device for all radiographic exams. The specimens, block of lead and penetrometer (containing 10 degrees, with an interval of 1 mm between them) were always placed on the phosphorous plate in the same position.

The images were saved in JPEG format and imported into the IMAGE PRO PLUS 6.0 software program (Macintosh computers, Maryland, USA), in which the pixel value of each degree of the penetrometer, and of the area of the diastema of the mandibular body were measured. A curve was prepared by means of a dispersion graph with pixel values versus the corresponding mmAl. Thus, the value of the diastema in mmAl was obtained.

The data were tabulated in the Microsoft Office Excel 2007 program in a spreadsheet for each radiographic image. The radiographic evaluations were made by a single, previously calibrated evaluator.