Effect of fluoxetine on induced tooth movement in rats

Giovana Carla Franzon Frigotto,[a] Cristiano Miranda de Araujo,[b] Odilon Guariza Filho,[c] Orlando Motohiro Tanaka,[d] Aline Cristina Batista Rodrigues Johann,[e] and Elisa Souza Camargo[f]
Curitiba, Paraná, Brazil

Introduction: Fluoxetine is a widely used antidepressant. Its various effects on bone mineral density are well described. The aim of this study was to evaluate the effect of fluoxetine on induced tooth movement. **Methods:** Seventy-two Wistar rats were divided into 3 groups: M (n = 24; 0.9% saline solution and induced tooth movement), FM (n = 24; fluoxetine, 10 mg/kg, and induced tooth movement), and F (n = 24; fluoxetine, 10 mg/kg only). After 30 days of daily saline solution or fluoxetine administration, an orthodontic appliance (30 cN) was used to displace the first molar mesially in groups M and FM. The animals were killed 3, 7, and 14 days after placement of the orthodontic appliances. The animals in group F did not receive induced tooth movement but were killed at the same times. We evaluated tooth movement rates, collagen neoformation rates by polarization microscopy, numbers of osteoclast by tartrate-resistant acid phosphatase, and trabecular bone modeling by microcomputed tomography of the femur. **Results:** The tooth movement rates were similar in groups M and FM at all studied time points ($P > 0.05$). The rate of newly formed collagen had a reverse pattern in groups M and FM, but the difference was not statistically significant ($P > 0.05$). There were significantly more osteoclasts in group FM than in group F on day 3 ($P < 0.01$). The trabecular spacing was significantly larger in group F compared with group M on day 14 ($P < 0.05$). **Conclusions:** Fluoxetine did not interfere with induced tooth movement or trabecular bone in rats. (Am J Orthod Dentofacial Orthop 2015;148:450-6)

Fluoxetine (eg, Prozac, Sarafem, Selfemra, and Rapiflux) is widely used in the treatment of depression and other psychological disturbances.[1] It belongs to the class of selective serotonin reuptake inhibitors and is involved in the increase of serotonergic neurotransmission in some areas of the brain via increased serotonin (5-hydroxytryptamine [5-HT]) release. Serotonin is a monoamine neurotransmitter synthesized in 2 phases from the essential amino acid tryptophan and the enzyme tryptophan hydroxylase. Serotonin release is related to behavioral, psychological, and cognitive functions.[2] These effects are mediated by 7 families of 5-HT receptors (5-HT1 through 5-HT7) and are regulated by the serotonin transporter 5-HTT, which is responsible for transporting 5-HT into the cell. Pharmacological agents such as fluoxetine antagonize 5-HTT and consequently potentiate serotonergic activity, relieving the symptoms of depression.[2,3]

The main components of the serotonergic system (eg, 5-HT receptors and 5-HTT transporters) are expressed in bone cells (osteoclasts and osteoblasts) and exert effects in bone cell activity.[4-7] Earlier studies have demonstrated that 5-HT regulates proliferation, differentiation, and activation of bone cells in vitro; therefore, blocking 5-HTT could affect bone metabolism. Selective serotonin reuptake inhibitors such as fluoxetine have been shown to decrease bone mineral density and, as a consequence, increase the risk of fractures[1]; however, the research results are conflicting.[1,8,9]

Orthodontic tooth movement is a complex process activated by biomechanical forces that cause bone resorption mediated by osteoclasts on the compression side and bone apposition mediated by osteoblasts on

From the School of Health and Biosciences, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil.
[a]Postgraduate student, Graduate Program in Dentistry, Orthodontics.
[b]Postgraduate student, Graduate Program in Dentistry, Orthodontics.
[c]Professor, Graduate Program in Dentistry, Orthodontics.
[d]Professor, Graduate Program in Dentistry, Orthodontics.
[e]Professor, Graduate Program in Dentistry, Histopathology.
[f]Professor, Graduate Program in Dentistry, Orthodontics.
All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
Funding support was received from Brazilian National Council for Scientific and Technological Development (CNPq), Brasília, Brazil.
Address correspondence to: Elisa Souza Camargo, Pontifícia Universidade Católica do Paraná, School of Health and Biosciences, Rua Imaculada Conceição, 1155, Prado Velho 80215-901, Curitiba, Paraná, Brazil; e-mail, escamargo@uol.com.br.
Submitted, November 2013; revised and accepted, April 2015.
0889-5406/$36.00

http://dx.doi.org/10.1016/j.ajodo.2015.04.031

the tension side.[10] Although some studies have indicated that fluoxetine has an effect on bone metabolism, none has investigated its effect on orthodontic tooth movement. We tested the hypothesis that the use of fluoxetine may affect tooth movement.

Therefore, the aim of this study was to evaluate the effect of fluoxetine administration on the tooth movement rate, bone remodeling, and trabecular bone microarchitecture during induced tooth movement in rats.

MATERIAL AND METHODS

This research was approved by the Committee of Ethics in Animal Research at Pontifícia Universidade Católica do Paraná in Brazil and was registered under protocol number 633.

The animals were 72 male Wistar rats (*Rattus norvegicus albinus*) born and housed at the university's animal facility. They were 9 weeks old and weighed 300 to 350 g. They were weighed with a precision scale (BG 4001; Gehaka, São Paulo, Brazil) on the first day of the experiment and every day thereafter until their death for drug dose adjustment. The animals were randomly divided into 3 groups: M (n = 24; saline solution and tooth movement), FM (n = 24; fluoxetine and tooth movement), and F (n = 24; fluoxetine only). In group M, the animals received daily intraperitoneal injections of 1 mL of 0.9% saline solution (LBS; Laborasa Indústria Farmacêutica, São Paulo, Brazil). Groups FM and F received daily intraperitoneal injections of fluoxetine of 10 mg per milliliter at 10 mg per kilogram, diluted in 53.3% propyleneglycol, 0.9% sodium chloride, and 0.1% sodium benzoate (Laboratório Farmacêutico da Pontificia Universidade Católica do Paraná, Curitiba, Brazil). The dose chosen is recommended for clinical use in humans.[11]

After 30 days of daily saline solution or fluoxetine administration, an orthodontic appliance was placed in each rat in groups FM and M. The orthodontic appliance was in accordance with the model proposed by Choi et al[12]; it consisted of a closed nickel-titanium coil spring (G&H Wire, Franklin, Ind) and a 0.010-in stainless steel ligature wire (Morelli, São Paulo, Brazil). The spring was fixed onto the maxillary right first molar and incisor. To provide more stability to the spring, the inferior incisors were abraded, and the superior incisors were secured together using Charisma composite resin (Heraeus, Hanau, Germany) after conditioning with 37% phosphoric acid (Condac 37; FGM, Joinville, Brazil) and an adhesive system (Adper Single Bond; 3M ESPE, St Paul, Minn). The reciprocal force produced by the spring was 30 cN.[13] The force magnitude was calibrated using a tension gauge (Haag-Streit, Koeniz, Switzerland). The orthodontic appliance caused the first molar to move mesially. To install the devices, the animals were sedated with intramuscular injections of tiletamine/zolazepam (50 mg/kg of Zoletil; Virbac Brasil Indústria e Comércio, Jurubatuba, Brazil).

The administration of saline solution and fluoxetine continued until the animals were killed by intraperitoneal injection of sodium pentobarbital (100 mg/kg; Thiopentax, Cotia, Brazil) at 3, 7, or 14 days after placement of the orthodontic appliances. The animals in group F were killed at 33, 37, or 44 days after the start of drug administration. A 9-mL blood sample was collected from the rats in groups F and FM via cardiac puncture into anticoagulant-free serum-separation tubes (Labor Import, São Paulo, Brazil). To confirm the administration of fluoxetine, drug plasma levels were determined by high performance liquid chromatography at Laboratório de Análises Clínicas Lanac (Curitiba, Brazil).

The right hemimaxilla of each animal was removed, dissected, and fixed in 10% formaldehyde for 24 hours and then decalcified in 5% EDTA for 3 months. The specimens were processed and embedded in paraffin at the Experimental Pathology Laboratory at Pontificia Universidade Católica do Paraná. After that, 2 adjacent 4-μm-thick cross-sections (for picrosirius red staining and tartrate-resistant acid phosphatase [TRAP] staining) were cut starting from the cervical third in the apical direction; then, 60 μm of tissue were skipped, and 2 more cross-sections were cut. The procedure was repeated for a total of 5 times on the mesiobuccal root of each specimen.

To evaluate and compare the microarchitecture of trabecular bone of the fluoxetine-treated animals, the left femur was removed, dissected, and frozen at a temperature of −20°C and later evaluated by microcomputed tomography (micro-CT). This analysis was performed on the femur to allow for standardization of the evaluated areas across the samples. In addition, intraperitoneal injection of fluoxetine has a systemic effect, with equal impact on all bones.

The distance between the maxillary right first molar and central incisor on the same side, before placement of the orthodontic device (initial measurement) and after the rats were killed (final measurement), was measured with a digital caliper (Absolute; Mitutoyo, Kawasaki, Japan). The rate of induced tooth movement was calculated using the following formula.

$$\text{Rate of induced tooth movement } (\%) = (\text{initial measurement}/\text{final measurement} - 1) \times 100$$

For the histologic analysis, in the picrosirius red-stained sections, the distal area of the mesiobuccal