Effect of Raloxifene on Periapical Lesions in Ovariectomized Rats

João Eduardo Gomes-Filho, PhD, Marcelo Tadahiro Wayama, MSc,* Rita Cássia Menegati Dornelles, PhD,† Edilson Ervolino, PhD,† Gilberto Aparecido Coclete, PhD,‡ Paulo Carvalho Tobias Duarte, MSc,* Guilherme Hiroshi Yamanri, MSc,* Carolina Simonetti Lodi, PhD,§ Eloi Dezan-Júnior, PhD,* and Luciano Tavares Angelo Cintra, PhD**

Abstract

Introduction: The aim of this study was to evaluate the effect of raloxifene (RLX) during progression of periapical lesions in ovariectomized (OVX) rats. **Methods:** Female Wistar rats were OVX or subjected to sham surgery and received vehicle or RLX by gavage for 90 days. The treatment groups were as follows: sham surgery and treated with vehicle (SHAM-veh), OVX and treated with vehicle (OVX-veh), and OVX and treated with RLX (OVX-RLX). During treatment, the pulp of lower first molar was exposed to the oral environment for induction of periapical lesion that was analyzed 7 or 30 days after procedure. Blood samples were taken from jugular vein for measurement of estradiol, and the mandibles were removed and prepared for radiographic, histopathologic, histometric, and immunohistochemical analysis. **Results:** Estradiol plasma concentration showed hypoestrogenism in OVX rats. The histopathologic analysis of the OVX/RLX group was similar to that of the SHAM-veh group, whereas OVX-veh group showed larger periapical lesions with more intense inflammatory response and more cells positive for tartrate-resistant acid phosphatase. Radiographically, the groups were similar, but lesions on day 7 were smaller than lesions on day 30. **Conclusions:** The results suggest that hypoestrogenism potentiates the progression of periapical lesions, and such condition was reversed by treatment with RLX. *(J Endod 2015;41:671–675)*

Key Words

Bone metabolism, estrogen, ovariectomized, periapical lesion, raloxifene

From the *Department of Endodontics, Araçatuba School of Dentistry, Universidade Estadual Paulista, São Paulo, Brazil; †Department of Basic Sciences, Araçatuba School of Dentistry, Universidade Estadual Paulista, São Paulo, Brazil; ‡Department of Radiology, Araçatuba School of Dentistry, Universidade Estadual Paulista, São Paulo, Brazil; §Department of Pediatrics, Araçatuba School of Dentistry, Universidade Estadual Paulista, São Paulo, Brazil.

Address requests for reprints to Dr João Eduardo Gomes-Filho, Endodontics, Araçatuba School of Dentistry, Univ. Estadual Paulista, R. José Bonifácio, 1193, 16015-050 Araçatuba, São Paulo, Brazil. E-mail address: jegomesfilho@yahoo.com.br

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.11.027

The growing elderly population has led to increasing interest in the treatment and prevention of diseases associated with the aging process (1). Pathologic oral conditions are adversely affected by some consequences of aging, such as osteopenia or osteoporosis promoted by postmenopausal hypoestrogenism (2, 3). This interest has encouraged a large number of investigators to conduct studies on animal models of the disease processes related to aging (2–4).

Periapical lesion starts with inflammation and necrosis of the dental pulp. Bacterial growth reaches the root canal system, recruiting inflammatory cells and osteoclasts promoting apical bone resorption (5). Bone metabolism is also regulated by systemic and local factors interacting to specific cells such as osteocytes, osteoblasts, and osteoclasts (6). Therefore, inflammatory mediators, growth factors, and hormones such as estrogen can influence the behavior of these cells (7).

Low estrogen concentration in plasma produces changes in many parts of the body, including oral tissues. Teeth and periodontitum are susceptible to changes in estrogen levels (8, 9) because that influences alveolar bone resorption (10), and its deficiency aggravates osteopenia and apical periodontitis (11).

Many therapies have been studied that aimed to treat and prevent the conditions resulting from estrogen deficiency. However, some of these treatments involving hormone replacement therapy have been associated with side effects such as bloating and breast tenderness (12) or even breast and uterine tumors (13). Raloxifene (RLX) was approved for the treatment and prevention of osteopenia and osteoporosis in estrogen deficiency condition, especially in postmenopausal women, because it is an oral selective estrogen receptor modulator that has estrogen mimicking effect in some organs. It has also been shown to increase bone mineral density and reduce the incidence of bone fractures (14). Moreover, it does not activate estrogen receptors in breast or uterine tissues (15); consequently, it has not been associated with tumors because of its specific receptor activity. Therefore, RLX is indicated in patients with a history of breast and/or endometrial neoplasms or other factors that contraindicate hormone replacement therapy (16).

Although the effects of RLX on bone metabolism have been highlighted, there are no studies evaluating the effects of RLX on periapical lesions in animals with hypoestrogenism. Therefore, the aim of the present study was to evaluate the progression of periapical lesions in ovariectomized (OVX) rats treated with RLX.

Materials and Methods

Animals

Forty-eight female Wistar rats (6 months of age) were distributed into groups: sham surgery plus vehicle treatment with 7 days (SHAM-veh 7d) or 30 days of pulp exposure (SHAM-veh 30d), OVX plus vehicle treatment with 7 days (OVX-veh 7d) or 30 days of pulp exposure (OVX-veh 30d), and OVX plus RLX with 7 days (OVX-RLX 7d) or 30 days of pulp exposure (OVX-RLX 30d). The experimental procedures were approved by the institutional ethics committee (Ethics Committee on Animal Use–Univ Estadual Paulista - 00799-2012).

Estrous Cycle

Samples of cells from the vaginal surface were collected with the use of a swab to determine the phase of the estrous cycle. The samples were extended in a histologic slide that was examined by use of optical microscope (Leica Microsystems, Wetzlar Germany) according to the technique of Long and Evans (17). Female rats with regular cycle (12 days) were randomly distributed into the experimental groups. Female rats with irregular estrous cycle were excluded from the study.

Ovariectomy

Rats were subjected to ovariectomy or sham surgery. The animals were anesthetized with ketamine (75 mg/kg, Vetaset; Fort Dodge Animal Health Ltd, São Paulo, Brazil) and xylazine (25 mg/kg, Coopazine; Coopers Ltd Brazil, São Paulo, Brazil) by intraperitoneal injection. An abdominal incision was made to expose the distal portions of the uterine tubes, and the ovaries were removed in the OVX groups. In the SHAM-veh group after the incision, the ovaries were exposed and repositioned. All animals had their incisions closed with sutures and received an intramuscular dose of antibiotics (1 mL/kg, Pentabiotic Veterinary; Fort Dodge Animal Health Ltd).

RLX or Vehicle Treatment

The treatment started after 10 days of ovariectomy or sham surgery to allow the reduction of estrogen in plasma. Vehicle (distilled water 0.3 mL) or RLX (Sigma-Aldrich, Munich, Germany) (1 mg/kg in 0.3 mL distilled water) was administered daily by gavage for 90 days (2, 3, 18).

Periapical Lesion Induction

Under general anesthesia, the right and left mandibular first molars had their pulp exposed to the oral environment with the aid of carbon drill bur (Drill Long Neck Ln; Maillefer Dentsply, Catanduva, Brazil). Access opening, 0.1 mm in diameter, was performed on days 60 and 83 of treatment with RLX or vehicle, enabling the lesion analysis on days 30 and 7, respectively.

Blood Sample Collection

Under general anesthesia, blood was collected from the jugular vein (19) and centrifuged (3000 rpm for 20 minutes at 2°C), and the plasma was stored in a freezer at –20°C for measurement of estradiol. The animals were killed by anesthetic overdose, and the mandibles were removed for radiographic, histopathologic, histometric, and immunohistochemical analysis. The uteri were also removed and weighed.

Plasma Estradiol Level Measurement and Uterus Weight

Plasma estradiol concentration was measured in duplicate by using a Biomedicals estradiol kit by radioimmunoassay (Costa Mesa, CA). The minimum detectable dose of estradiol was 5.0 pg/mL, and the intra-assay value was 3.9%. Uterus weights were determined on a precision balance (Mettler Toledo, Barueri, SP, Brazil).

Radiographic Analysis

The mandibles were fixed in 4% formaldehyde for 24 hours. The radiographs were obtained by using a digital x-ray machine (Dabi Atlante Spectro 70/10; Ribeirão Preto, São Paulo, Brazil) with calibration at 70 kV and 10 mA, 12 pulses, and 40 cm focal length. The radiation incidence was focused perpendicular to the film-object plane. A phosphorus-activated optical plate (Digora, Soredex; Orion Corporation, Helsinki, Finland) and an aluminum penetrometer (6063 alloy) were used to capture images (24 bits in TIFF format [tagged image file format]). The areas of the periapical lesions at the root apices of the molars were quantified in pixels by using DIGORA for Windows 1.51 software. The data were converted to square millimeters by using 1 mm^2 = 256 pixels, as determined by assaying a standard of known area (20).

Histopathologic, Histometric, and Immunohistochemical Analysis

Mandibles were decalcified in 10% EDTA for 60 days and subjected to conventional histologic processing. Semi-serial sections (4 μm) were performed in the laterolateral direction, allowing sectioning of the mandibular first molar in its longitudinal axis. Sections were stained with hematoxylin-eosin or submitted to immunohistochemistry by using an indirect immunoperoxidase technique for tartrate-resistant acid phosphatase (TRAP) (primary antibody goat anti-TRAP SC 30832; Santa Cruz Biotechnology, Santa Cruz, CA) following previously described protocol (21). Histopathologic, histometric, and immunohistochemical analyses were performed by certified histologist (E.E.) who was blinded regarding the experimental groups.

Histologic analysis was conducted by using the following parameters: nature and extension of inflammation, presence and extension of necrosis, vasculature state, and pattern of cellularity of dental and periodontal tissues.

For histometric analysis of the size of the periapical lesion, the periapical lesion area associated with the distal root of the mandibular first molar was measured by using Leica Microsystems Software (Leica, Wetzlar, Germany). The area of the periapical lesion was calculated by rounding up the lesion boundary, considering the outer external surface of the cementum, periodontal ligament, and the outer surface of the alveolar bone, and it was expressed in square millimeters. The measurement was conducted in 5 equidistant sections to the root canal, and just the largest area was selected.

The osteoclast amount was analyzed in the histologic section used for histometric analysis. The perimeter was calculated by contouring the boundary of the periapical lesion with the aid of Leica Microsystems

TABLE 1. Serum Estradiol Levels, Uterus Weight, Radiographic Density, Periapical Lesion Areas, and TRAP-positive Cells According to the Groups

Experimental groups	Serum estradiol levels (pg/mL)	Uterus weight (g)	Radiographic density (pixel)	Periapical lesion (mm^2)	TRAP (cells/mm^2)
SHAM-veh 7d	331.2 ± 146.9^b	0.54 ± 0.12^b	82.84 ± 4.49^a	0.37 ± 0.04^a	4.48 ± 1.60^a
OVX-veh 7d	146.9 ± 39.8^a	0.25 ± 0.15^a	82.58 ± 5.03^a	0.57 ± 0.06^b	7.68 ± 1.34^b
OVX-RLX 7d	136.0 ± 45.1^a	0.21 ± 0.06^a	83.24 ± 5.23^a	0.31 ± 0.07^a	3.34 ± 1.46^a
SHAM-veh 30d	818.0 ± 159.9^c	0.59 ± 0.16^b	77.04 ± 5.24^b	1.26 ± 0.24^c	5.55 ± 2.07^a
OVX-veh 30d	125.8 ± 31.1^a	0.19 ± 0.14^a	77.05 ± 5.13^b	2.55 ± 0.45^d	9.63 ± 5.15^c
OVX-RLX 30d	119.9 ± 34.4^a	0.19 ± 0.06^a	78.82 ± 5.12^b	1.39 ± 0.26^c	4.45 ± 1.03^a

In a single column, different superscript letters indicate statistical difference.