Journal of Cranio-Maxillo-Facial Surgery 41 (2013) 581–585

Contents lists available at SciVerse ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Isotretinoin effect on the repair of bone defects – A study in rat calvaria

Henrique T.R. de Oliveira [a], Roberta D. Bergoli [b], Wâneza D.B. Hirsch [b], Otacílio L. Chagas Jr. [c,*], Cláiton Heitz [d], Daniela N. Silva [b,e]

[a] Dentistry Department, Fundação para Reabilitação das Deformidades Cranio Faciais (FUNDEF), Lajeado, RS, Brazil
[b] Oral and Maxillofacial Surgery Post-Graduate Program, School of Dentistry, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil
[c] Department of Oral and Maxillofacial Surgery and Maxillofacial Prosthodontics, Bone Repair Research Group, School of Dentistry, Universidade Federal de Pelotas (UFPEL), Pelotas, RS, Brazil
[d] Surgery Department, School of Dentistry, PUCRS, Porto Alegre, RS, Brazil
[e] Universidade Federal do Espírito Santo (UFES), Vitória, ES, Brazil

ARTICLE INFO

Article history:
Paper received 29 August 2012
Accepted 26 November 2012

Keywords:
Bone and Bones
Vitamin A
Hypervitaminosis A
Isotretinoin

ABSTRACT

Background: Isotretinoin is a vitamin A derivative, indicated for the treatment of patients with severe acne, which shows several side effects on bone metabolism.
Objective: This study analyzed the process of bone repair in rats receiving 7.5 mg/kg/day of oral isotretinoin.
Methods: Thirty-three male albino Wistar rats, at approximately 60 days of age, were randomly assigned to control ($n = 15$) and experimental ($n = 18$) groups. Only the experimental group underwent oral isotretinoin therapy. In both groups, a 2-mm cavity was established in the calvarium of each animal. The animals were euthanize 21, 28 and 90 days postoperatively. The parietal bone was removed and the surgical specimens underwent histological examination. Computed histomorphometry allowed the measurement of the total area of bone defects and the proportion of newly formed bone at the different observation time points.
Results: In the experimental group, the results, expressed as mean percentage of newly formed bone, were: 25.37% (±9.14) at day 21; 41.78% (±7.00) at day 28; and 57.51% (±11.62) at day 90. In the control group, the results were: 17.10% (±9.23) at day 21; 34.42% (±7.70) at day 28; and 48.49% (±16.40) at day 90.
Conclusion: These results enabled us to conclude that isotretinoin promoted acceleration in the process of new bone formation in rat calvaria, although this increase was not statistically significant.

1. Introduction

Isotretinoin, or 13-cis-retinoic acid, is a vitamin A derivative currently used as the drug of choice in the treatment of severe acne that is unresponsive to the usual antimicrobial therapy. Since the introduction of the drug, in September 1982, isotretinoin has become an important therapeutic agent for dermatologists, who should, therefore, be aware of its side effects (Azulay et al., 1985). Excessive retinoid intake may cause unpleasant and even worrying acute or chronic clinical alterations, which can vary from individual to individual in a dose-related fashion. Side effects are similar to those observed with hypervitaminosis A, including dry lips as well as more severe alterations, such as reduced bone mineral density – increasing the risk of fracture, liver lesions, inhibition of bone growth, in addition to laboratory alterations, such as an increase in cholesterol and triglycerides, and alterations in liver enzymes and alkaline phosphatase (Azulay et al., 1985; Sampaio and Pimentel, 1985).

Concerning bone tissue, some reports have shown that excessive retinoid intake has site-specific effects, such as a reduction in bone density of 9% at the Ward triangle, hyperostotic changes or calcification of tendons and ligaments, premature closure of epiphyses (Leachman et al., 1999; DiGiovanna et al., 2004), increased bone resorption, increase in the number and size of osteoclasts, decrease in osteoid surface, and deterioration of cartilage (Frankel et al., 1986). However, the effects on cranial bones have yet to be determined.

* Corresponding author. R. Santa Cruz, 1948 A/504 – Centro, Pelotas, RS, CEP: 96015-710, Brazil. Tel./fax: +55 53 32224305.
E-mail address: otaciliochagasjr@gmail.com (O.L. Chagas).

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http://dx.doi.org/10.1016/j.jcms.2012.11.030

Taking into consideration that acne vulgaris is the most common skin disorder among adolescents and adults, that isotretinoin is the drug of choice in the treatment of severe acne, and that isotretinoin-related craniofacial alterations remain unclear, patients undergoing this therapy have become an object of great concern among professionals in the area of Oral and Maxillofacial Surgery and Trauma, since bone tissue is one of the main tissues approached in several branches of this specialty.

This study evaluated the effects of daily administration of isotretinoin on the repair of bone defects made in rat calvaria, using a therapeutic dose corresponding to that used for skin disorders such as severe acne in humans.

2. Materials and methods

This study was approved by the Research Ethics Committee of the School of Dentistry, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), and the Ethics Committee for the Care and Use of Animals – PUCRS.

Thirty-three male albino rats (*Rattus novergicus albinus*, *Wistar*), at approximately 60 days of age and mean weight of 250 g, were used in the study. The animals were randomly assigned to two groups:

Control group – 15 rats without isotretinoin administration were divided into three subgroups (five rats per group) according to the time point for observation of bone repair: 21, 28 and 90 postoperative days;

Experimental group – 18 rats receiving daily isotretinoin for 30 days prior to the surgical procedure were divided into three subgroups (six rats per group) according to the same observation time points used for the control group, with continuous isotretinoin administration until euthanasia. The animals in the experimental group were weighed before treatment and then weekly for drug dosage adjustment.

2.1. Oral administration of isotretinoin

Isotretinoin, available as a powder, was diluted in sunflower oil to produce oral suspensions. Isotretinoin powder and sunflower oil were placed in dark glass containers and stored in a refrigerator until their use in the experiment. Each suspension was prepared immediately before administration due to isotretinoin high sensitivity to air, heat, and light, especially as a suspension. Suspensions were prepared by injecting sunflower oil, using an adjustable dosage syringe, into a container with powder isotretinoin until the desired concentration of 7.5 mg/kg body weight was provided (Ferguson et al., 2005), shaking the container for a better dilution of the content. Isotretinoin was administered orally, by the gavage procedure, using a 2-mL syringe and a metal cannula suitable for this purpose.

2.2. Surgical procedure

The rats were anaesthetized intraperitoneally with 50 mg/kg 10% ketamine hydrochloride (0.05 mL/100 g) and 5 mg/kg xylazine hydrochloride (0.025 mL/100 g). Trichotomy was performed in the calvarium in the region between the ear auricles. Surgical access was established by means of a linear coronal cutaneous incision (skin, subcutaneous tissue and periosteum) of approximately 1.5 cm between the ears. A cavity was made in the right parietal bone, lateral to the median sagittal suture, using a low-torque electric motor, with 2-mm spherical drills, corresponding to the size of the defects produced. Perforations were made under abundant irrigation with 0.9% saline solution, with a rupture of the outer and inner cortex of the calvarium, with no meningeal lesion.

The periosteum, subcutaneous tissue and skin were then sutured with 4–0 mononylon simple running suture. For postoperative pain management, the animals received analgesia with paracetamol (80 mg/kg) intramuscularly on the first three postoperative days.

The animals were euthanized 21, 28 and 90 days after the surgical procedure, through continuous inhalation in an isoflurane chamber.

2.3. Preparation of samples

An osteotomy was performed in the right parietal bone, with a tronco-conical drill (702), at low drilling speed, under continuous irrigation with saline solution, at a distance of at least 4 mm from the cavity, completed with the use of a straight chisel, aiming to minimize possible damage to the bone due to drilling friction. The complete operated area was removed and surgical specimens were then prepared.

The specimens obtained were placed into labeled glass containers containing 10% buffered formalin solution. After 48-h fixation, the specimens were decalcified in 5% nitric acid solution, changed daily, for 2–4 days, according to bone thickness. After decalcification, a 6-μm thick section was obtained from the central region of the defect of each specimen, at the greater diameter (2 mm), and stained with hematoxylin–eosin (HE).

2.4. Image capture procedure and histomorphometry

Histological slides underwent microscopic examination using a computer-assisted image processing and analysis system (Image J software, version 1.41, Media Cybernetics Inc., Bethesda, MD). The microscope image was captured with a fixed-focus camera (Sony-CCD-Iris Color Video Camera, model DXX-107a, Sony, Tokyo, Japan) attached to a PC-based workstation (1.8 GHz processor, 128 MB RAM, 40 GB hard drive, Compaq Computer Corporation, Houston, TX), at 40× magnification. The images were then transformed into an electrical analog signal and transmitted to the computer screen, where the image was digitized, constituting a set of pixels (1 pixel = 6.5 μm). After the images were saved as JPEG files (a total of 33 histological slides corresponding to all control and experimental groups), they were submitted to histomorphometry. Using the image processing and analysis software, we could measure the total area of the defect and the area of new bone formation by moving the cursor over the image to draw its outline, thus resulting in the proportion of newly formed bone at the different observation time points (Fig. 1). The values obtained from each newly formed trabecular bone specimen were transferred to a table, in which total bone formation was recorded and calculated for each slide analyzed. All these values were entered on definitive Excel spreadsheets (Microsoft Corporation, Redmond, WA) and submitted to statistical analysis using the SPSS software (Statistical Package for the Social Sciences, version 11.5; SPSS Inc., Chicago, IL).

After image acquisition, the outline of the area of bone defect and areas of newly formed bone was drawn with the mouse. The value of these areas, as μm^2, was quantified using the image processing and analysis software, the proportion of newly formed bone being calculated as follows: area of newly formed bone/total area of defect.

The nonparametric Mann–Whitney test was used to compare the percentage of new bone formation between experimental and control groups (intergroup test). The nonparametric Kruskal–Wallis test was used to compare observation time points (21, 28 and 90 postoperative days) within the same group (intragroup test: