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Research Article

Radiographic Study of the Post-Extraction Socket: Local Effect of Alendronate and *Aloe vera*

Aguzzi Alejandra[1]*, Virga Carolina[1], De Leonardi Gabriela[1], Briner Garrido Michelle[2] and Moro Cecilia[1]

[1]*Oral Pathology, National University of Cordoba School of Dentistry, Argentina*

[2]*Department of Oral Pathology, Radiology and Medicine, University of Missouri-Kansas City*

***Corresponding Author:** Aguzzi Alejandra, Oral Pathology, National University of Cordoba School of Dentistry, Argentina.

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Abstract

Introduction: The maintenance of bone mass in the jaws has always been a concern for dental professionals, as it is essential for teeth and implants support.

Objective: To study the effect of local administration of Alendronate sodium (AL) and Aloe vera (VA) on tissue regeneration of the post-extraction socket of the lower first molars in rats.

Materials and Methods: The experimental rats were divided into groups, depending on the drug combination they would receive. Four groups were formed: Control (saline solution), Alendronate sodium (0.5mg/Kg of weight), *Aloe vera* (at 70% concentration) and *Alendronate sodium+Aloe vera* (0.5mg/Kg of weight + 70% *Aloe vera*). The rat's lower first molars were extracted, then gelatin sponges soaked in the experimental drugs were placed into the extraction socket. The handling of the animals was carried out following the norms established by SECyT according to the Institutional Committee for the care and use of laboratory animals. Radiographs of the jaws were taken at 0, 15, 30 and 60 days with a radiovisiograph (Sirona Xios) and analyzed with the ProPlus Image Software version 4.1. Data were analyzed by ANOVA test.

Results: In the radiographic study, at 30 days a significant increase in bone mineral density was evidenced in the AV group compared to the C group ($p < 0.01$).

Conclusions: The data suggest that the process of new bone formation would be more accelerated in the *Aloe vera* and Alendronate sodium+*Aloe* vera groups given the higher optical density recorded in the 30-day stage in contrast to the Alendronate sodium alone and the control groups.

Keywords: Alendronate; *Aloe vera*; Bone Remodeling

Introduction

There are different clinical situations where the removal of a tooth is needed. After the extraction, alveolar bone resorption of the remaining ridge occurs, leading to atrophy of the alveolar bone and soft tissue collapse. This situation may cause aesthetic and functional problem, even prevent the placement of a dental implant due to the lack of adequate bone volume [1,2].

In the same way, we know that the success of a dental implant is determined not only for its longevity and strong osteointegration, but also by the aesthetic result obtained when it has been functionally rehabilitated. This largely depends on the bone volume available before the implant placement, which is why minimizing the loss of the alveolar ridge, after dental extraction becomes a fundamental requirement for an optimal result [3,4]. In this context, knowledge of the bone remodeling process after extraction is essential for a successful implant planification.

Maintaining the bone volume, inhibition of the bone resorption and bone regeneration have been topics largely studied for the last couple of decades. With this objective in mind, various drugs, material and techniques have been developed for this purpose. Among these therapeutics used to intervene in bone mineralization and turnover we find calcium, parathyroid hormone (PTH), vitamin D, calcitonin, bisphosphonates, sodium fluoride (NaF), sodiumfluorophosphate (MFP), sex hormones and the most promising drugs denosumab and teriparatide [5,6].

Bisphosphonates are potent inhibitors of bone resorption. Alendronate sodium (AL) is the most widely used drug of this class and acts in bone remodeling by reducing bone resorption in a dose-dependent manner. It works mainly by inhibiting recruitment and promoting apoptosis of osteoclasts, while stimulating osteblastic activity [7,8]. Due to its poor oral absorption, attempts have been made to increase its bioavailability trough different parental routes. It has been proven that subcutaneous administration has the same efficiency than intravenous administration, in regards of drug bioavailability [9]. Alternative drug administration methods such as local administration or other drugs capable of achieving an efficient osteogenesis are needed [10,11].

Aloe vera (AV) is a natural element known as a biogenic stimulant and modulator of hormonal activity during wound healing. The chemical composition of AV consists of aloin, aloe-emodin, barbaloin, amino acids and anthraquinones. Antraquinones have several beneficial properties such as anti-inflammatory, analgesic and antimicrobial effects, as well as anesthetic and antioxidant effects. Another material found in AV, aloe-emodin, is also known to promote tissue healing and recovery process. It could also trigger growth stimulation of fibroblasts and osteoblasts in the tissue [12,13].

General objective

To study the effect of local administration of Alendronate and *Aloe vera* on an experimental model in rats.

Specific objectives

- To analyze radiographically the effects of Alendronate and *Aloe vera* in the process of bone healing at 0, 15, 30 and 60 days after extractions.
- To compare the effects and results of the drugs in a rat model of alveolar bone healing.

Material and Methods

This *in vivo* experimental study was approved by the Institutional Committee for the Care and Use of laboratory Animals. The handling of the animals was carried out in accordance with the standards established by Secretary of Science and Technique (SECyT) following the regulations of this committee (Res. HCD 674/09 National University of Cordoba School of Medicine).

Male Wistar rats were used as they are reproduceable and easy to handle animals [14]. They were kept in the animal facility of the physiology department of the National University of Cordoba School of Dentistry, in collective cages (up to 5 rats per cage). These cages measured 427 mm wide, 267 mm length and 180mm tall, giving a total area of 820 cm^2. The rats were supplied with balanced food (portions of 35 to 40 grams per rat a day) and water as desired, at a temperature of 22 to 26 degree Celsius, with a light-dark cycle of 12 hours each during the duration of the experiment.

Drugs preparation: Highly purified absorbable gelatin sponges of animal origin (porcine), of neutral pH, in the form of 3mmx3mmx3mm cubes, sterilized by gamma radiation, were used. For the experimental group, these sponges were soaked with Alendronate solution (GADOR®) (0.5mg/Kg of weight) and *Aloe vera* gel 70% diluted [15]. This *Aloe vera* gel is a colorless mucilaginous gel obtained from the parenchymal cells of fresh leaves [16]. To mix the *Aloe vera*+Alendronate, 1ml of each substance was placed in a sterilized dappen dish. The alendronate solution and *Aloe vera* gel were gently mixed together using a spatula, ensuring a homogeneous mixture. For the control group, a saline solution was used.

Surgical procedure performed on the experimental animals (first molar extractions): 64 male Wistar rats were used. Special care was taken so that the weight of the animals was 90 +/- 15 grams, since overweight rats tend to develop hypercementosis making the extraction procedure more difficult to perform. Four groups of 16 rats each were formed: control (C), *Aloe vera* (AV), Aledronate (AL) and *Aloe vera*+Aledronate (AV+AL). For the control group, the gelatin sponge soaked in saline solution was placed inside the extraction sockets. For the AL group, the same technique was used, but the sponge was soaked in Alendronate solution (0.5 mg/Kg of weight). For the AV group, a sponge with 70% *Aloe vera* ger was used. Lastly, for the AL+AV group, the sponge was soaked in a mix of Alendronate and *Aloe vera*.

The technical approach to perform the surgical maneuvers in the oral cavity of the rat, previous investigations [17,18] were considered as references. A special stretcher was prepared that allowed the animals to be kept in dorsal decubitus position. The animals were anesthetized with a ketamine/xylazine solution in a ratio of 8mg/1.28mg, respectively, for every 100g of body weight.

Once anesthetized and after asepsis of the surgical field, the rats were placed on the stretcher. The dental extractions of the mandibular first molars were done, and the soaked sponges were placed inside the extraction alveolus, accordingly to the experimental group they belonged.

At the end of the experiment, the animals were euthanized under general anesthesia, by intracardiac injection of potassium chloride, at 0, 15, 30 and 60 days after the procedure. After the euthanasia of the animals, the jaws were resected (necropsy) and were placed in a sterile bottle and fixed with 10%formaldehyde.

Radiographic studies: Radiographic images of the jaws were taken using a radiovisiograph (Sopix SOPRO software), for all groups at each experimental time, and then analyzed using Image Pro Plus Software version 4.5.0.29 from Media Cibernetics, designed to work with Olympus confocal microscopes. The images were taken placing the mandibular lingual aspect over the sensors with the x-rays directed perpendicular to it. The same position, exposure time (0,2 seconds) and distance (1cm) were used for all images taken. The software was able to measure optical density (gray scale) and record numerical values from the area on interest.

The digital radiographs of the rat's jaws evaluated in regards their gray level at the tooth extraction sites. The gray scale available in this software ranges from 0 (black) to 255 (white). The gray value, that represented the density of the region of interest, was quantified (mg Ca^{++}/cm^2) using an aluminum pattern.

The area of interest on the alveolar bone was determined to be located from above the superior aspect of the inferior alveolar canal to the alveolar crest, and from the mesial and distal aspects of the extracted tooth.

Statistical analysis

The statistical and data analysis and the graphs shown in this study were done utilizing Graph Pad Prism 2.0, GraphPad Software® (San Diego, California USA). Analysis of Variance (ANOVA test) was carried out using utilizing the treatment performed in each group (C, AL, AV, AL+AV) and treatment times (0, 15, 30 and 60 days). A Tukey test was also used to comparing factors such as days and groups to determine how they differed. A significant p value of <0,05 was used.

Results

At day 0, every group showed similar results.

At day 15, there was no significant differences between the groups, although the control group recorded values somewhat lower than the rest of the experimental groups (p = 0,06) (Figure 1) (Graph 1).

Figure 1: Radiographs at day 15.

Graph 1: Distribution of the gray values of each experimental group at day 15.

At day 30, the values reported for the control and Alendronate groups were similar. The results of the *Aloe vera* +Alendronate and the *Aloe vera* were also similar. However, significant differences were found between the C and AV groups) p < 0,001 (Figure 2) (Graph 2).

Figure 2: Radiographs at day 30.

Graph 2: Distribution of the gray values of each experimental group at day 30. *p < 0,01 AV-C.

At day 60, the gray value intensity distribution of all groups shows a similar result (Figure 3) (Graph 3).

Figure 3: Radiographs at day 60.

Graph 3: Distribution of the gray values of each experimental group at day 60.

Graph 4 shows the behavior of the different experimental groups at different times, summarizing all previously described.

Graph 4: Evolution of the gray values intensity of each group.

Discussion

Bone repair is a natural process in which the body seeks to replace lost bone tissue with the same or similar characteristics [19]. The objective of this research was to study the effect of the local administration of Alendronate and *Aloe vera* on the tissue repair process in extraction sockets of lower first molars of rats through a radiographic study. In this investigation, the mandibular first molars were selected as it is commonly extracted and easy to standardize. The upper molars in the Wistar rat are in contact with the maxillary sinus and the incisors are essential for feeding and quality of life, so that was an additional reason to select mandibular first molars. According to Gottardello., *et al*. molar extraction in rats is more appropriate than other animals, due to the short repair period. In their manuscript, after performing the dental extractions, they sutured the wound to prevent bleeding. However, in our study, as in Biasotto., *et al.* study, the wound was compressed with gauze for a couple of minutes to control bleeding [20,21].

Bisphosphonates are potent inhibitors of bone resorption. Alendronate is the most commonly used frug and acts on the bone remodeling process by reducing bone resorption in a dose-dependent manner. Based on previous studies, the AL formulation was prepared to be locally applied. Studies on cell viability determined two concentration ranges: one (0 to 10 µg/ml), in which cell viability is not affected and another (10 to 20 µg/ml), where said viability begins to be affected. This variability made it possible to use the optimal dose of this drug without toxicity risks. Additionally, the dose of 0.5 mg/Kg weight of AL was chosen, in accordance with the doses suggested in the literature [22].

A pharmacokinetic study of the AL parameters shows that the main disadvantages is its poor oral absorption from the gastrointestinal tract, as less than 1% is absorbed. Due to this reason, attempts have been made to increase its bioavailability by different parenteral routs. It is equally effective if administered subcutaneously or intravenously, in regards to its bioavailability. By these routes, it is estimated that more than 50% of the drug is available for its incorporation into the bone matrix [23]. It is worth mentioning that in the dental field osteonecrosis of the jaws is a potential adverse reaction due to intravenous administration at high doses in the presence of oral sepsis.

That is why alternative routes of administration were needed, such as a local or systemic route that would be capable of achieving effective osteogenesis. Because of this, investigators have been trying to find suitable carriers that can provide an osteoconductive matrix that would impart all needed properties required for implantation at the working site in order to improve the AL delivery avoiding side effects [24,25]. Usually, the challenge for new drugs

administrations systems is to achieve adequate bioavailability and safety, which is why AL was administered locally in this study as an alternative rout of administration with the aim of reducing adverse effects.

On the other hand, natural compounds have been considered as alternative or complementary tools of modern medicine that provide an effective therapeutic option [26]. In bone repair and regeneration, several natural polymers have shown bioactivity and osteoconductivity [27]. *Aloe vera* is a natural material known as a biogenic stimulator and modulator of hormonal activity during wound healing. A compound found in *Aloe vera*, aloe-emodin, is known to promote tissue healing and recovery processes. It could also trigger tissue growth stimulation of fibroblasts and osteoblasts [28,29].

To achieve the objectives proposed in this work, radiographic studies were carried out in order to measure the optical density (OD), in this way the gray intensities of the radiographs were assessed in relation to the absorption coefficient of the sample; A curve was obtained that contemplates the pixel intensity of the X-ray, so that the amount of bone mineral could be observed and the bone mineral density (BMD) could be quantified.

The BMD analysis was performed, which allowed us to establish the quality of bone formed in the post-extraction alveolus, through an increase in the radiopacity of this area as time progressed. Radiographically, an increase in BMD was determined in the experimental groups. It was observed that after 15 days there is evidence of an increase in BMD in favor of the *Aloe vera* group (AV) and AL+AV, with significant differences for AV with respect to the control (C) at 30 days ($p < 0.01$). While at 60 days the values are balanced.

An animal study agrees with our results; In it, the lower right incisors of male Sprague-Dawley rats were extracted and a sponge treated with acemannan (polysaccharide extracted from *Aloe vera* gel) was placed in the alveolus. Acemannan-treated groups had higher bone mineral density and faster bone healing compared to untreated controls [30].

On the other hand, Viera-Negrón., *et al.* analyzed BMD in male rats after subcutaneous administration of AL, using doses of 1 mg/kg 3 times a week. Radiographic bone density was determined at 3 points: mesial, apical and distal. At 28 days, LA-treated rats had significantly higher radiographic bone density than the other groups ($p < 0.01$) [31].

Kwang-Won P., *et al.* investigated the effect of AL released locally from biphasic calcium phosphate (BCP) microspheres. The capacity for bone regeneration in a rat tibial defect model was evaluated by radiographic imaging. Radiographic studies revealed that bone growth in the AL-treated group increased at a dose of 5 mg ($p < 0.05$). However, there were no significant differences in bone mineral density when using a dose of 1 mg of AL compared to the control group [32].

The present investigation, on the other hand, did not show significant differences between control and AL, which could be due to the local route of administration used, which may have hindered diffusion through cell membranes to reach the site of action (bone tissue). due to the physical-chemical characteristics of AL. Remember, most drugs are weak acids or bases, and the pH of body fluids at the sites of absorption or excretion is critical to their pharmacokinetics. Weak acid or weak base drugs come in two forms; one water-soluble ionized and the other non-ionized fat-soluble, and the passage through biological membranes is conditioned by its degree of ionization, if it is a weak acid or base, the pH of the medium where it is found and the pKa of the drug [33].

Due to the scarcity of studies that explain the possible beneficial action of AV in bone repair, additional studies are needed to determine its exact mechanism of action on bone metabolism, since apparently, they would act by increasing cell proliferation and the formation of extracellular matrix, which would favor the start of the bone remodeling phase from osteoprogenitor mesenchymal cells.

Conclusion

The results achieved allow us to assert that the local administration of *Aloe vera* increased bone mineral density, evidenced radiographically, by higher values than Control and Alendronate groups at all experimental times, presenting significant differences at 30 days ($p < 0.01$). with respect to the Control group. The combination of Alendronate and *Aloe vera* showed a similar response to the administration of *Aloe vera* alone, suggesting that the benefits were primarily produced by *Aloe vera*.

The local administration of *Aloe vera* and *Aloe vera* + Alendronate proved to be effective in improving the bone healing conditions of the post-extraction socket, of the lower first molar, in male rats. However, further studies are needed to expand upon these conclusions.

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