Alveolar Bone Loss: A Shorter-Time Study Model in Mice

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Summary

Mucoperiosteal flap surgery (MPS) of the mouth is conducted in several clinical situations. However, MPS triggers a resorption process that leads to alveolar bone ridge loss. Rodents are routinely used in studies evaluating alveolar bone loss (ABL), and the experimental time involved in these experiments is usually 21 days. This study aims at establishing whether the alveolar bone resorption area differs between 10 and 21 days after MPS in mice mandibles. MPS was performed in the vestibular aspect of the left mandible (LM) of 20 male CF1 mice *Mus domesticus*. The right mandible (RM) was used as control. Animals formed identical groups for each experimental time. Animals were euthanized 10 days (ten days group, TG) or 21 days (twenty one days group, TWG) after the surgical procedure. All mandibles were hemisectioned, cleaned and stained for stereomicroscopic inspection. Digital images were obtained and the alveolar bone loss area measured (mm^2) using image analysis software. The results demonstrate that a significant loss is observed in the left mandible (LM) (Student's *t* test, $p<0.01$), as compared to the RM, in both groups. No statistically significant difference was observed in the ABL area ($p>0.05$) between TWG and TG. This investigation leads to the conclusion that it is possible to reduce experimental times when using the MPS model in mice.

Introduction

In dentistry, surgical access to the alveolar bone is conducted in several clinical situations and in various specialities. Yet, when the periosteum is separated from the alveolar bone, osteoclastic activity is stimulated, which ends in a resorptive phase that leads to bone ridge loss (*Staffileno et al., 1962; Staffileno et al., 1963; Ranjford and Costish, 1968; Yaffe et al., 1994*). This loss, results from surgical exposure of the bone, is an undesired outcome of the repair process, and many studies have been conducted in an attempt to find a way to avoid or minimize it (*Yaffe et al., 1995; Yaffe et al., 1997, Yaffe et al., 2000; Binderman et al., 2001; Kaynak et al., 2003)*.

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Rodents are routinely used as experimental models in the evaluation of alveolar bone loss (ABL), since the animals have a short life (*Broobank, 1990*). Other advantages of this animal model include the ease in handling and reproduction, the low costs in acquiring and maintaining the animals, apart from the anatomic similarity between molars and oral structures of mice and those of humans (*Jordan, 1971*).

Yaffe *et al.* (1994) observed the occurrence of a regional accelerated phenomenon (RAP) that results from full-thickness flap surgeries. The authors reported a striking resorption of the cortical bone three weeks after the surgery.

Based on that study, numerous studies have been conducted to assess ABL or to test drugs that could prevent ABL within a 21-day experimental period in rodent mandibles (*Yaffe et al., 1995; Yaffe et al., 1997; Binderman et al., 2000; Kaynak et al., 2003*). Nevertheless, Grevstad and Boe (1995) conducted an investigation and observed that the proliferation of osteoclasts reached a maximum level 10 days after mucoperiosteal flap surgery (MPS).

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This investigation was motivated by the possibility to conduct experiments using the ABL model in rodents over a shorter period. This study aims at establishing whether the alveolar bone resorption area differs between 10 and 21 days after MPS in mice mandibles.

Materials and Methods

All animal care was in accordance with the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Committee of Ethics in Research of the Lutheran University of Brazil.

Twenty 3-month-old male CF1 mice (*Mus domesticus*) weighing on average 30g were used in this study. Animals were obtained from the colonies maintained by FEPPS (Fundação Estadual de Produção e Pesquisa em Saúde). Standard pellet feed (Nutrival, CR-1, Nutrival Nutrients, Curitiba, PR, Brazil) and distilled water were available ad libitum. Sterilized bedding (Vet-Sul, Porto Alegre, RS, Brazil) was provided. The experiment was conducted under proper ventilation and all mice were kept under standardized light (12-h dark/light cycles) and temperature (~20°C) conditions. The mice were housed in sterilized plastic cages (Beira Mar, São Paulo, SP, Brazil) with an iron cover during the experiment. All animals underwent MPS, and euthanasia was conducted at two distinct experimental times: 10 days (TG) and 21 days (TWG) after the surgery.

Experimental procedure

Mice were weighed and received an intramuscular injection (1.0-ml/kg) of anesthetics composed of a 1:1 of ketamine (100g/l) (Dopalen, Agribrands do Brazil, Paulina, Brazil) and aqueous solution of 2(2,6-xylidine)-5,6-dihydro-4-H-1,3-xylazine hydrochloride (2%) (Rompun, Bayer S.A., São Paulo, Brazil). Mice were appropriately placed on a surgical table in order to access oral structures (*Rivaldo and Padilha, 2007*). The marginal gingiva was separated from the bone after incision and then immediately repositioned without suture (*Yaffe et al., 1994; Yaffe et al., 1995; Yaffe et al., 1997, Yaffe et al., 2000; Binderman et al., 2001; Kaynak et al., 2003; Rivaldo et al., 2005*). The mucoperiosteal flap surgery was conducted on the vestibular aspect of the lower left molars in a procedure that took approximately 40 seconds, while the right aspect was used as the control. The animals were given only water in the first 24 hours after surgery, to prevent flap displacement. This experiment was undertaken using a humane technique, avoiding pain and distress of animals. All procedures and euthanasia were undertaken under anaesthesia in order to assure animal welfare.

Using a surgical microscope (M900, D F Vasconcelos, São Paulo, Brazil), mandibles were hemisectioned, dissected and cleaned with a microbrush using sodium hypochlorite (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, Brazil) to remove all organic material. Specimens were stored in formaldehyde 10% for 12 hours (*Tatakis and Guglielmoni, 2000; Al-Rasheed et al., 2003; May and Tatakis, 2004*). Hemimandibles were stained with 1% toluidine blue to mark the exposed root area, the enamel and bone limits, and the cement. After, images were digitally captured (Pixera System™, San Jose, USA) in a stereomicroscope (Stemi SV6 Zeiss™, Jena, Germany) under 3.2x magnification. Specimens were maintained at a standardized position, with vestibular and lingual cusps at the same level (*Rivaldo et al., 2007*).

Analysis of bone loss

Area of the exposed root (mm^2) was measured blind by a trained researcher, by using image analysis software UTHSCA Image Tool version 3.0. Two measurements of each specimen were conducted at a 1-week interval to assess reproducibility. Intra-examiner reproducibility data and inter-experiment data, obtained according to the method proposed by Bland and Altman (1986) varied between -0.0208 and 0.012 mm^2.

On the vestibular aspect, the ABL area measurements were done on the first molar, while on the lingual aspect first and second molars were used. ABL was measured using the reference points proposed by Tatakis and Guglielmoni (2000) and modified by

Hilgert *et al.* (2002). The results are presented as square millimeters (mm^2).

Statistical analysis

The Student's *t* test for independent samples was used to evaluate the difference between the mean ABL area on the left mandible vestibular aspect (LV; operated), and the right mandible vestibular aspect (RV, control), as well as the difference between the ABL areas of the control groups when RV, LV, left lingual aspect (LL) and right lingual aspect (RL) were compared. Significance level for all results was 5%.

These analyses were conducted using the SPSS software 12.0 for Windows (SPSS Inc. Illinois, USA).

Results

ABL was observed after MPS. ABL area on the operated mandible side (LV) exhibited significant loss when compared to the control mandible side (Student's *t* test; $p<0.05$). There were no differences in terms of weight loss between the animals groups (data not shown). There were no differences in respect to ABL between TG and TWG groups, $p>0.05$ (Figure 1).

Table 1. Vestibular and lingual areas (mm^2), right and left side, 10 days (TG) and 21 days (TWG) after surgery.

	Mean (±SD)
RV (TG)	0.28 (± 0.01)
RV (TWG)	0.41 (± 0.17)
LV (TG)	0.59 (± 0.19)
LV (TWG)	0.60 (± 0.23)
RL (TG)	0.93 (± 0.28)
RL (TWG)	0.93 (± 0.26)
LL (TG)	0.98 (± 0.21)
LL (TWG)	1.15 (± 0.28)

SD: standard deviation

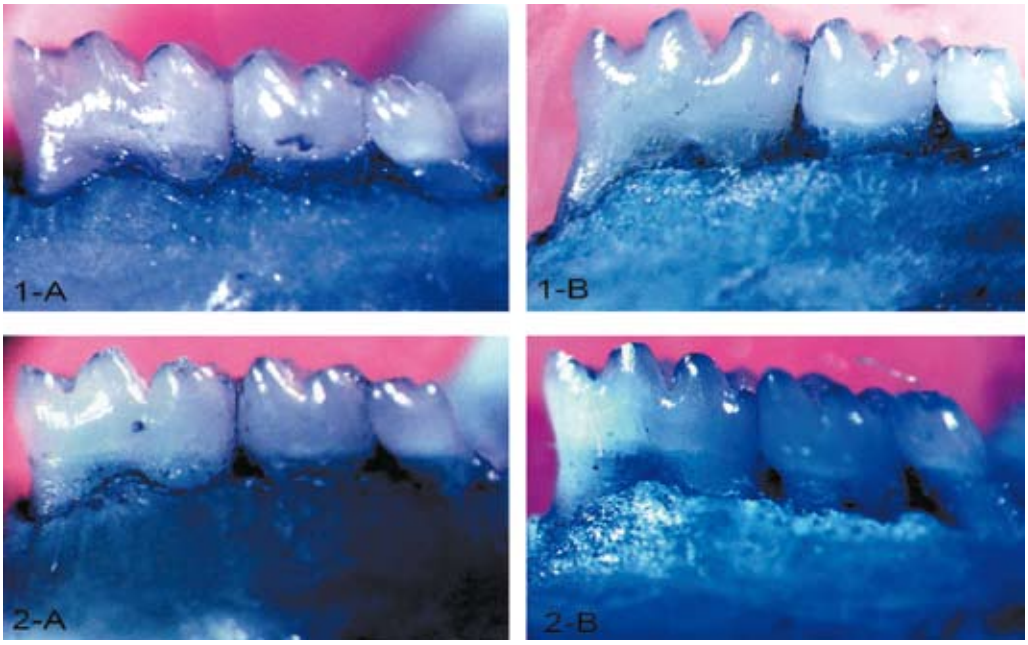

Figure 1. Non-operated mandible side lingual aspect at 10 days (1A) and 21 days (2A) and operated mandible side lingual aspect at 10 days (1B) and 21 days (2B) after MPS.

There were no statistically significant differences in mean ABL area of the mandible (for RV, LV, RL or LL) at 10 days after surgery compared with after 21 days. (Table 1)

Discussion

Several studies have reported that resorption of alveolar bone developed 3 weeks following mucoperiosteal flap surgery in rats (*Yaffe et al., 1994; Yaffe et al., 1995*). We have also reported that alveolar bone loss is observed in mice 3 weeks after surgical procedure (*Rivaldo et al., 2005*). In this study we have shown that experimental time can be reduced in mice to 10 days without influencing the outcome of the experiment. We use this timing in another investigation where we access the effect of raloxifen on alveolar bone resorption after MPS. Similarly, no statistically significant differences were observed in the comparison of results obtained for these experimental times (data not shown).

Animal experimentation is a fundamental component of biomedical research. There is a concern about increasing costs of purchasing and caring for laboratory animals. The time of an experiment is a critical component of those costs. Shortening the time of an experiment not only reduces the laboratory cost but also provides an improvement of experimental design by reducing the number of animals necessary to accomplish longer experiments. With this in mind, the results obtained in the present study are very important in the investigation of ABL in mice, as they show it is possible to reduce the time of keeping animals for such an investigation.

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