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Observations on experimental marginal periodontitis in rats

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Objective: The purpose of this study was to assess periodontal destruction following experimentally induced marginal periodontitis in rats by ligatures over a 60-day observation period. The extent to which the physiological movement of teeth influenced the effect of the ligatures was also examined. In addition, two methods for measuring bone loss in the defleshed jaw were compared.

Methods: Thirty-five male Sprague-Dawley rats (SD) were divided into five groups. Marginal periodontitis was induced by ligatures on the second maxillary molars. Rats were killed after 15, 30, and 60 days. Rats in the control group were killed on day 1 and day 60. Bone loss was determined with two different methods on the buccal and palatinal surfaces of the defleshed jaw. In the first method, the distance of the cementoenamel junction (CEJ) from the alveolar bone crest (ABC) was measured at different sites; in the second method, the area of the exposed root surface of the molars was measured.

Results: Comparison of the control groups from day 1 and day 60 using both measuring methods showed significant differences in bone loss. In the area where the ligature was located, test rats exhibited significantly greater bone loss than control rats. Comparison of control rats from day 1 with test rats from day 15 showed that the increase in bone loss between the groups within the area of the ligature was significantly greater than outside it. The age-dependent bone loss increases over the entire observation period of 60 days. The ligature-induced bone loss increased most from day 1 to day 15; on days 30 and 60, slighter increases in bone loss were observed.

Conclusions: The application of this model can only be recommended for short (≤15 days) observation periods. The distance method should be preferred to the area method.

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In studies on the etiology or therapy of multifactorially determined diseases, examining the influence of one individual factor is made easier by minimizing or standardizing the influences of the other factors. Animal experiments are well suited to such cases. In rats, periodontal disease (1–14), diabetes (15), and cardiovascular diseases such as high blood pressure (16) or stroke (17, 18) can be experimentally induced.

Earlier studies showed that as long as a rat lives – but especially pronounced within the first 100 days postpartum – remodelling processes occur in the alveolar bone that greatly effect the positional relation between molars and the margin of the alveolar bone. Through the apposition of root cement, the root length increases. Attrition decreases the height of the molar crowns, particularly in the first 100 days after birth. The continual eruption of teeth associated with this leads to an increase in the distance between the cementoenamel junction (CEJ) and the alveolar bone crest (ABC) of up to 0.8 mm (19).

However, a lengthening of this distance also occurs as part of periodontal destruction. This distance is also frequently used as proof of marginal periodontitis on defleshed skull preparations

(5, 6, 20, 21). On defleshed bone, bone loss caused by destructive periodontal processes cannot be distinguished from physiologically determined remodelling and rebuilding processes.

The purpose of this study was to assess periodontal destruction following experimentally induced marginal periodontitis in rats by ligatures over a 60-day observation period. The extent to which the physiological movement of teeth influenced the effect of the ligatures was also examined. In addition, two methods for measuring bone loss in the defleshed jaw were compared.

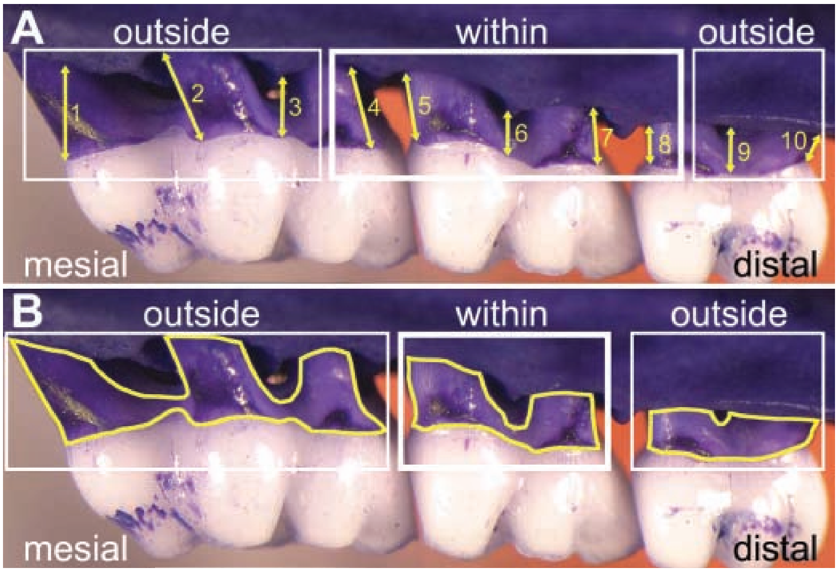

Fig. 1. (A) Distance method. Overview of maxillary posterior dentition. Right: oral with sketched-in distances measured. 30 ×. (B) Area method. Overview of maxillary posterior dentition. Right: oral with sketched-in areas measured. 30×.

Materials and methods

Rats

Thirty-five male Sprague-Dawley rats (SD) from Charles River Inc. (Sulzfeld, Germany) were placed in an animal laboratory (12–24 h light/dark cycle: light on 7.00 a.m. to 7.00 p.m.; temperature: 22°C; humidity: 40–60%) at the age of 4–4.5 months and divided into five groups: rats without ligatures were killed on day 1 (group C1; baseline) and day 60 (group C60; control) of the experiment. Ligatures were placed in group L15, L30, and L60 rats on day 1. These rats were killed on days 15 (L15), 30 (L30), and 60 (L60). The rats were given water and dry food (Ssniff Spezialdiäten, Soest, Germany) ad libidum. The experiment was registered and approved by the local animal care committee.

Experimental periodontitis model

Under general anesthesia [mixture of acepromazine maleate (Sanofi-Ceva, Düsseldorf, Germany), xylazine hydrochloride (BayerVital, Leverkusen, Germany) and ketamine hydrochloride (ASTA Medica, Frankfurt, Germany)] injected intraperitoneally at a dosage of 0.1 ml/100 g body weight, silk ligatures (strength 5/0, Resorba, Nürnberg, Germany) were tied around the neck of the second maxillary molar teeth using a technique that was previously described by other authors (9, 11, 13). After the rats were killed by decapitation, the maxillae were biologically and chemically defleshed (22), and stained with methylene blue (24).

Microscopic examination of periodontal bone loss

Using a light microscope (Olympus Optical Co., Hamburg, Germany) with a color video camera mounted on it and a computer, the oral and buccal surfaces of the posterior maxillary dentition were recorded in a standardized manner (30 × magnification). Standardization of the recordings was assured by fixed reference points on the rat jaws. Using the image analysis software analySIS 3.0 (Soft Imaging System, Münster, Germany), bone loss as determined by the two methods of measurement was calculated.

Distance method — Ten oral and 10 buccal distances were measured from the CEJ to the ABC (Fig. 1A). The oral distances 1, 2, 3, 9, 10 and the buccal distances 1, 2, 8, 9, 10 lay outside the placement zone of the ligature; the oral distances 4, 5, 6, 7, 8 and the buccal distances 3, 4, 5, 6, 7 lay within the placement zone of the ligature. Repeated measurements for this method yielded a relative coefficient of variation of 1.45%.

Area method — The areas of the stained oral and buccal root surfaces (six areas measured per jaw) were measured (Fig. 1B). Areas 1 and 3 lay outside the placement zone of the ligature, and area 2 within. Repeated measurements for this method yielded a relative coefficient of variation of 1.03%.

Statistical methods

The general linear model was used for the statistical analysis. The dependent variables were the distance CEJ–ABC and the area of the exposed root surfaces; the independent variables were group assignment (control, ligature), time point, the factor area/ligature with two steps (with/without), and the factor location with two steps (buccal/oral). In addition to the main effects, the possible interactions were tested for significance.

Because the distances and areas differ greatly in size, meaning that the changes they undergo with time can also vary greatly, they were standardized with a z-transformation ($z_i = (x_i - \text{mean})/\text{SD}$ and averaged according to groups.

Results

Evidence of age-dependent bone loss

Control rats from day 1 (C1) and day 60 (C60) were compared in terms of bone loss (Figs 2A and B). For both measuring methods, the C60 rats exhibited significantly higher bone loss

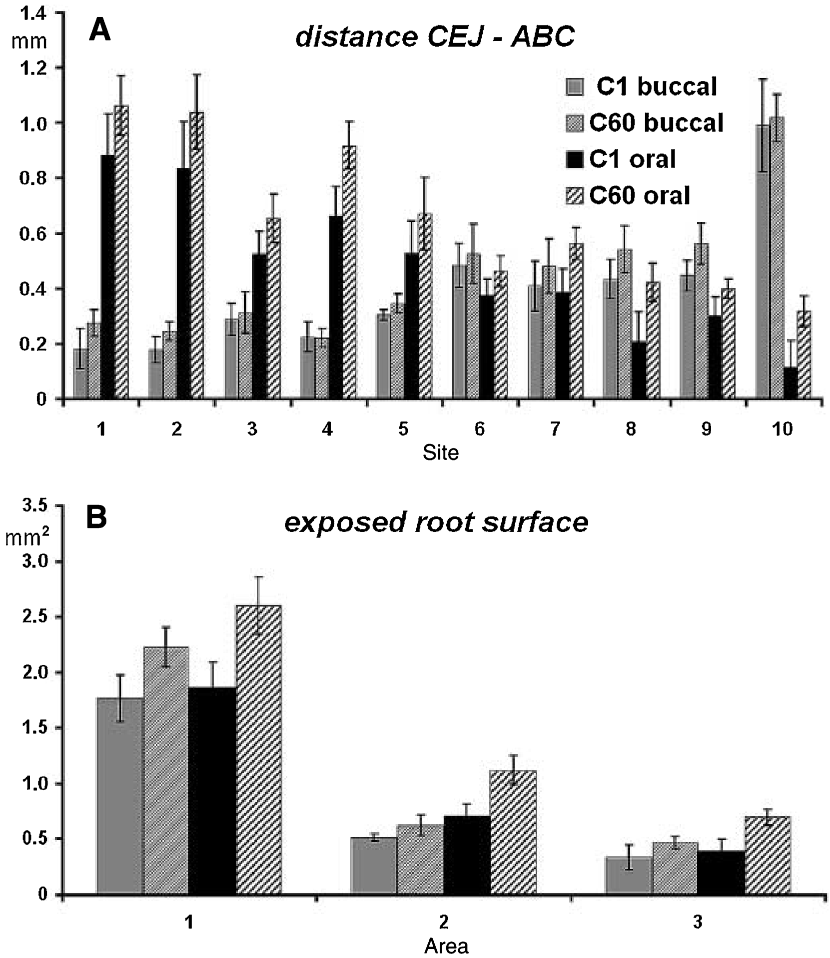

Fig. 2. (A) distance method: cementoenamel junction–alveolar bone crest (CEJ-ABC)-distance in the control rats from day 1 (C1) and control rats from day 60 (C60) groups; buccal and oral distances. (B) area method: areas of the exposed buccal and oral root surface in groups C1 and C60.

than did the C1 rats. Bone loss over time was more pronounced on the oral than on the buccal surfaces. The area method also showed that the increase in exposed root surfaces was greater in the region of the first molar than in that of the second and third (Fig. 2B).

Evidence of experimentally induced bone loss

Control rats (C60) and rats of the same age with ligatures (L60) were compared. The buccal and oral distances measured showed significantly greater bone loss in the L60 rats in the region of the ligatures ($p = 0.019$ for the distance method) than the corresponding distances in the C60 rats. The buccal differences were somewhat greater than the oral (Fig. 3A). In contrast, the area method revealed no significant differences between the exposed root surface areas in L60 vs. C60 rats ($p = 0.13$) (Fig. 3B). In the test and control groups (L60 and C60), the distances and areas within the ligature effect zone were also compared with the distances and areas outside this zone. For both methods within the ligature effect zone, significant differences were found between groups C60 and L60 ($p = 0.025$ for the distance method, $p = 0.03$ for the area method); again, buccal differences were more pronounced than oral. In contrast, no significant differences between groups were found for either method outside of the ligature effect zone. In addition control rats (C1) and rats with ligatures (L15) were compared. The increase in both CEJ–ABC distance and exposed root surface area from baseline to day 15 was significantly greater within the ligature effect zone than outside it ($p = 0.0025$ for the distance method; $p = 0.0205$ for the area method) (Fig. 4).

Progression of bone loss with time

Both measuring methods demonstrated significantly greater ligature-induced bone loss between days 1 (C1) and 15 (L15) within the ligature effect zone than outside it. Subsequently, a stagnation in the progression of bone loss up to day 60 was observed, indicating a diminishing effect of the ligatures. On the oral surfaces, age-dependent bone loss – which was more pronounced orally than buccally – is responsible for the rapid equalization of the alveolar bone level between the surfaces within and outside of the ligature effect zone (Fig. 4).

Discussion

Physiological bone loss

The comparison of control rats from day 1 (C1) and day 60 (C60) shows a significant increase in the CEJ–ABC distance for all sites measured. This observation agrees with that of other authors. In 1940, Hoffman and Schour observed rats from birth to an age of 1050 days. Within this time period, they noted an increase in the CEJ–ABC distance of up to 0.8 mm (19). The 60-day CEJ–ABC distance increase measured in this study was *c.* 0.1–0.15 mm. Belting *et al.* obtained similar results (25). Earlier experiments with sterile (germ-free) rats also found this distance increase with age (5, 8). This makes clear that the processes responsible for this phenomenon are not in any way related to the onset and development of periodontal disease. Rather, it is the result of growth and bone remodelling processes occurring in the jaw as a whole, in addition to rapid occlusal attrition of the teeth caused by the consumption/mastication of food, the consequence of which is movement of the molars in the occluso-disto-buccal direction (19, 23).

The increasing CEJ–ABC distance caused by these physiological bone remodelling processes in rats is a major

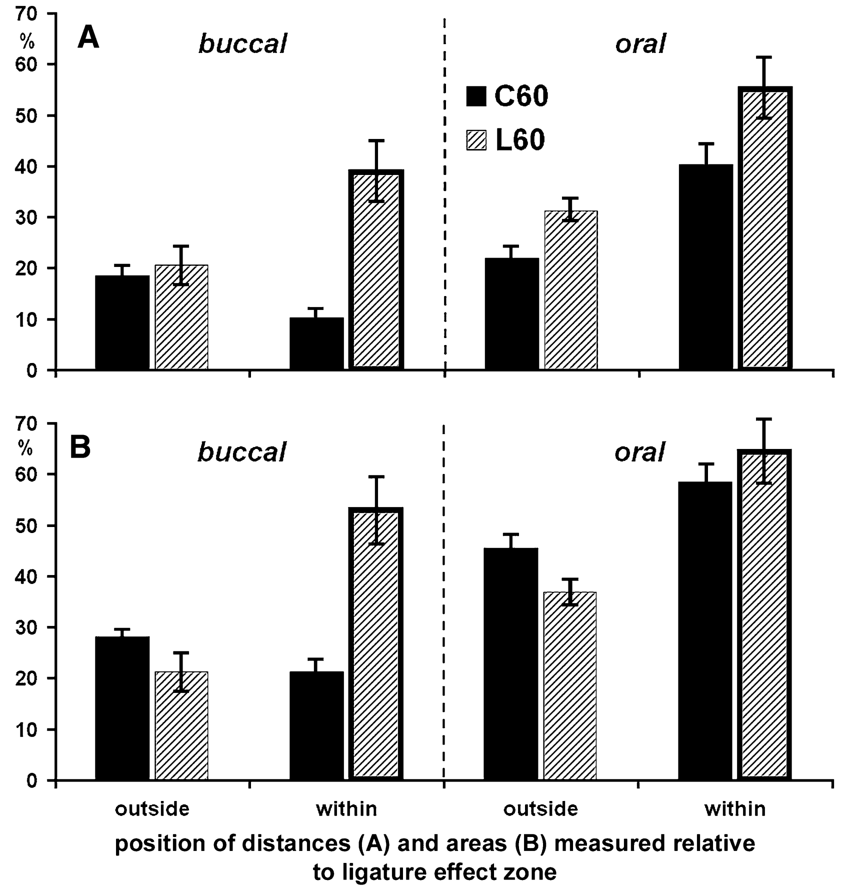

Fig. 3. (A) distance method. Percentage increase in the cementoenamel junction–alveolar bone crest distance (day-1 values = baseline) in the control rats from day 60 (C60) and the rats with ligatures from day 60 (L60) groups. (B) area method. Percentage increase in the area of exposed root surfaces (day-1 values = baseline) in groups C60 and L60. One mean value from the values measured within and one mean value from the values measured outside of the ligature effect zone were calculated and presented for the buccal and oral surfaces. Thickly outlined bars represent ligature sites.

disadvantage of this animal model for experimentally induced marginal periodontitis. The measured bone loss on the defleshed jaw is the result from physiological bone remodellation and ligature induced bone loss (in ligature sites) or the result from physiological bone remodellation alone (control sites). Physiological and ligature induced bone loss can not be separated on the defleshed jaw. Control groups are necessary to estimate the portion of physiological bone loss on entire bone loss.

Comparison of rats without ligatures from day 1 and day 60 exhibits a similar increase in bone loss in areas within the ligature effect zone and areas outside the ligature effect zone. Furthermore there are only slight differences in L60- and C60-rats in bone loss outside the ligature effect zone. It can be assumed that quantity of physiological bone loss is not affected by existence of ligature. Effect of ligature is restricted to adjacent structures; systemic effect of ligature has not been described in previous articles. Therefore, we conclude that the physiological bone loss within and outside the ligature effect zone has the same magnitude. Thus the missing control-groups on days 15 and 30 is not crucial for interpreting the results of this study.

Ligature-induced bone loss

Ligature-induced bone loss is particularly visible on day 15 (200–300 μm increase in the CEJ–ABC distance in the ligature effect zone). Outside the area in which the ligature is located, the CEJ–ABC distance increases consistently due to the previously mentioned age-dependent bone remodelling processes (50–100 μm on day 15, *c.* 150–200 μm on day 60). Possibly, this is a factor which contributes to the diminution of the ligature effect after day 15, since the movement of the teeth away from the sulcus necessarily also distances the ligatures from their site of efficacy.

Adams *et al.* (26) and Kennedy and Polson (27) made similar observations in experiments with monkeys; they, too, induced marginal periodontitis by placing ligatures and found that the subsequent bone loss occurred chiefly in the first 2 weeks, and slowed consistently up to the end of the experiment at 10 weeks. Nyman *et al.* (28) documented the same phenomenon in beagles. Ligature-induced bone loss was highest 21 days after ligature placement. On day 28, a remission of osseous bone remodelling processes was observed. Nyman *et al.* pointed out that the position of the ligature relative to the gingival margin played a large role in the efficacy of the ligature. The deeper the ligature lay subgingivally, the greater was the damage it caused. This led them to conclude that the processes involved were a rapidly progressing, acute inflammatory reaction rather than a gradually progressing, chronic lesion. Schroeder and Lindhe (29) and Heijl *et al.* (30) drew the same conclusions from experiments on dogs and monkeys in which periodontitis was induced via ligatures.

Rovin *et al.* (11) placed ligatures around the first mandibular molars in 6-month-old rats; half the rats were raised under sterile and half under conventional conditions. Over a period of 26 weeks, animals were killed at regular intervals. The epithelial attachment in sterile and conventional rats without ligatures remained at the level of the CEJ over the entire observation period; in the rats bearing ligatures, the epithelial attachment had receded to an average of 300 μm below the CEJ after 2 weeks. However, the authors assumed that this recession among rats raised under sterile

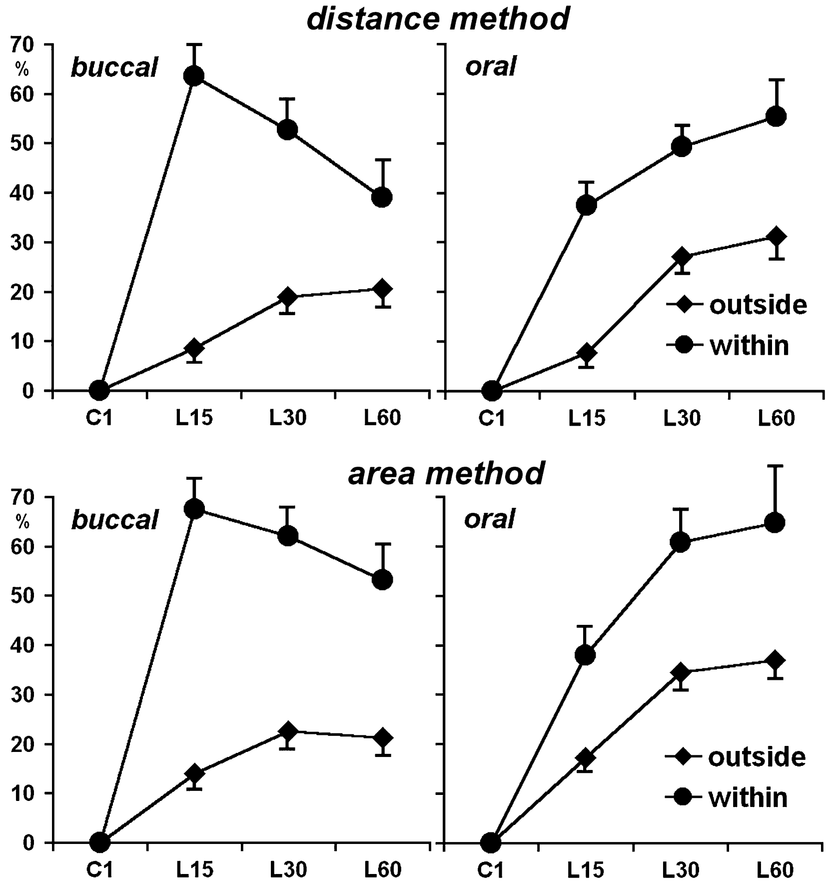

Fig. 4. Temporal progression of bone loss: percentage increase in cementoenamel junction–alveolar bone crest distance from day 1 to day 15, day 30 and day 60 in the corresponding groups with ligatures. One mean value from the values measured within and one mean value from the values measured outside of the ligature effect zone were calculated and presented for the buccal and oral surfaces.

condition was due to a suppressive effect of the ligature, since signs of inflammation were found only among the conventionally raised rats. Rats kept under sterile conditions also failed to exhibit bone loss – as similarly reported by Asman *et al.* in experiments with sterile rats (11, 31). Thus, a general mechanical effect of the ligature in the absence of a bacterial effect can be excluded as the instigator of the observed bone loss.

Our experiment does not make it possible to decide whether a shift in the equilibrium between bacterial attack and host defence follows an acute, short-term inflammation or if the tooth movement in the occlusal direction causes a 'migration' of the ligature out of the gingival sulcus. The ulceration of the epithelial barrier enables bacteria to migrate into deeper tissues (9, 13). It is conceivable that the effect of the ligature, which also consists in promoting plaque accumulation, is lost through the bone remodellation associated with the ligature's movement in the coronal direction.

Comparison of measurement methods

The distance method is widely used (5, 6, 12, 14, 20). In it, bone loss is taken as the distance from the cementoenamel junction to the alveolar bone crest as measured at exactly defined sites. The area method uses the exposed root surfaces areas as a measure of bone loss (1). In our experiment, both methods confirmed that even without the influence of ligatures, the CEJ–ABC distance increases with age. The area method demonstrates this even more clearly than the distance method. This can probably be attributed to the fact that the area method includes the entire exposed root surface and hence detects changes in bone level much earlier than does the distance method, which only measures certain distances on the root surface.

At day 15, ligature-induced bone loss can be assessed with both methods. Both methods show significant differences between groups C1 and L15. In comparing groups C60 and L60 to determine ligature-induced bone loss, the area method yielded no significant difference between groups, i.e. evidence of ligature-induced bone loss was not found. In contrast, the distance method detected significant differences between groups C60 and L60 ($p = 0.019$), providing evidence of a ligature effect. This contradiction can be explained as follows: if only the second molars and not the first and third are considered to lie within the ligature effect zone, then in the area method, the mesial and distal approximal surfaces of the third and first molars, respectively, will be erroneously evaluated. The distance method, however, by virtue of the greater number of distances measured per tooth, allows a much more exact division of regions into those located within and those outside of the ligature effect zone.

A further factor could be the root anatomy of the molars. The occurrence of bifurcations and a narrowing of the roots in the apical direction means that the area of exposed root surfaces does not increase linearly with alveolar bone loss, but instead lags behind the bone loss rate.

Conclusion

When working with defleshed rat skulls, control groups must consist not only of baseline animals, but also of same-age animals in order to distinguish physiological from experimentally induced periodontal alterations. If slight differences in bone loss exist between experimental groups, the osseous bone remodelling processes should be measured with the distance method instead of with the area method. An additional disadvantage of this rat model is the limited duration of the ligature's effect. For this reason, the model used here should only be applied with relatively short observation periods (≤15 days).

References

1. Burckhardt JJ, Gaegauf-Zollinger R, Schmid R, Guggenheim B. Alveolar bone loss in rats after immunization with Actinomyces viscosus. *Infect Immun* 1981;**31:** 971–977.
2. De Lima V, Bezerra MM, Alencar VB. Effects of chlorpromazine on alveolar bone loss in experimental periodontal disease in rats. *Eur J Oral Sci* 2000;**108:** 123–129.
3. Chang KM, Ramamurthy NS, McNamara TF, Genco RJ, Golub LM. Infection with a gram-negative organism stimulates gingival collagenase production in non-diabetic and diabetic germfree rats. *J Periodont Res* 1988;**23:**239–244.
4. Chang KM, Ramamurthy NS, Mc Namara TF *et al.* Tetracyclines inhibit *Porphyromonas gingivalis*-induced alveolar bone loss in rats by a non-antimicrobial mechanism. *J Periodont Res* 1994;**29:** 242–249.
5. Crawford JM, Taubman MA, Smith DJ. The natural history of periodontal bone loss in germfree and gnotobiotic rats infected with periodontopathic microorganisms. *J Periodont Res* 1978;**13:**316–325.
6. Evans RT, Klausen B, Sojar HT, *et al.* Immunization with *Porphyromonas* (*Bacteroides*) *gingivalis* fimbriae protects against periodontal destruction. *Infect Immun* 1992;**60:**2926–2935.
7. Golub LM, Evans RT, McNamara TF, Lee HM, Ramamurthy NS. A non-antimicrobial tetracycline inhibits gingival matrix metalloproteinases and bone loss in *Porphyromonas gingivalis*-induced periodontitis in rats. *Ann N Y Acad Sci* 1994;**732:**96–111.
8. Heijl L, Wennstrom J, Lindhe J, Socransky SS. Periodontal disease in gnotobiotic rats. *J Periodont Res* 1980;**15:**405–419.
9. Labelle RE, Schaffer EM. The effects of cortisone and induced local factors on the periodontium of the albino rat. *J Periodontol* 1966;**37:**483–490.
10. Navia JM. *Animal models in dental research*. Alabama: The University of Alabama Press, 1977: 467.
11. Rovin S, Costich ER, Gordon HA. The influence of bacteria and irritation in the initiation of periodontal disease in germfree and conventional rats. *J Periodont Res* 1966;**1:**193–204.
12. Ryder MI. Histological and ultrastructural characteristics of the periodontal syndrome in the rice rat. I. General light microscopic observations and ultrastructural observations of initial inflammatory changes. *J Periodont Res* 1980;**15:**502–515.
13. Sanavi F, Listgarten MA, Boyd F, Sallay K, Nowotny A. The colonization and establishment of invading bacteria in periodontium of ligature-treated immunosuppressed rats. *J Periodontol* 1985;**56:** 273–280.
14. Stahl SS, Dreizen S. The adaptation of the rat periodontium to prolonged feeding of pellet, powder and liquid diets. *J Periodontol* 1964;**35:**312–319.
15. Doxey DL, Cutler CW, Iacopino AM. Diabetes prevents periodontitis-induced increases in gingival platelet derived growth factor-B and interleukin 1-beta in a rat model. *J Periodontol* 1998;**69:** 113–119.
16. Kodavanti UP. The spontaneously hypertensive rat as a model of human cardiovascular disease: evidence of exacerbated cardiopulmonary injury and oxidative stress from inhaled emission particulate matter. *Toxicol Appl Pharmacol* 2000;**164:**250–263.
17. Eke A, Conger K, Anderson M. Histologic assessment of neurons in rat models of cerebral ischemia. *Stroke* 1990;**21:** 299–304.
18. Longa Z, Weinstein PR, Carlson S. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989;**20:**84–91.
19. Hoffman MM, Schour I. Quantitative studies in the development of the rat molar. II. Alveolar bone, cementum and eruption. *Am J Orthod* 1940;**26:**854–874.
20. Crawford JM, Taubman MA, Smith DJ. The effects of local immunization with periodontopathic microorganisms on periodontal bone loss in gnotobiotic rats. *J Periodont Res* 1978;**13:**445–459.
21. Samejima Y, Ebisu S, Okada H. Effect of infection with *Eikenella corrodens* on the progression of ligature-induced periodontitis in rats. *J Periodont Res* 1990;**25:** 308–315.
22. Piechocki R, Altner HJ. Makroskopische Präparations-technik. Teil 1: Wirbeltiere. *Spektrum.* Heidelberg: Akademischer Verlag, 1998: 76–79.
23. Sicher H, Weinmann JP. Bone growth and physiologic tooth movement. *Am J Orthod* 1944;**46:**109–132.
24. Romeis B. Mazeration von Skeletteilen. In: Böck P, ed. *Mikroskopische Technik*. München: Urban und Schwarzenberg, 1989, 318–319.
25. Belting CM, Schour I, Weinmann JP, Shepro MJ. Age changes in the periodontal tissues of the rat molar. *J Dent Res* 1953;**32:**332–353.
26. Adams RA, Zander HA, Polson AM. Cell populations in the transseptal fiber region before, during and after experimental periodontitis in squirrel monkeys. *J Periodontol* 1979;**49:**7–12.
27. Kennedy J, Polson AM. Experimental marginal periodontitis in squirrel monkeys. *J Periodontol* 1973;**44:**140–144.
28. Nyman S, Schroeder HE, Lindhe J. Suppression of inflammation and bone resorption by indomethacin during experimental periodontitis in dogs. *J Periodontol* 1979;**50:**450–461.
29. Schroeder HE, Lindhe J. Conversion of stable established gingivitis in the dog into destructive periodontitis. *Arch Oral Biol* 1975;**20:**775–782.
30. Heijl L, Rifkin BR, Zander HA. Conversion of chronic gingivitis to periodontitis in squirrel monkeys. *J Periodontol* 1976; **47:**710–716.
31. Asman B, Wijkander P, Hjerpe A. Reduction of collagen degradation in experimental granulation tissue by vitamin E and selenium. *J Clin Periodontol* 1994; **21:**45–47.