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# Alveolar bone resorption in a model of periodontitis in rats

## Animal model in periodontal research

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## Introduction

Periodontal disease is a chronic inflammatory disease characterized by destruction of gingival connective tissue and resorption of alveolar bone caused by periodontopathic bacteria. Inflammatory destruction of the periodontium can be spontaneous or induced experimentally in most mammalian species. The rat is a commonly used animal in experimental periodontitis studies as most normal or dental histological features are similar in rats and humans, except that rats have keratinaised sulcular gingival epithelium.

## Objectives

Aim of the study: to characterise a rat model of periodontitis for use in functional studies by analysis of alveolar bone loss in rats with experimentally-induced periodontitis.

## Material and Methods

1. Induction of periodontitis

- Periodontitis was induced in male Wistar rats (250-300g) by intragingival injection of 1ml Lipoppolysaccharide (10mg/ml) from Salmonella typhimurium (Sigma-Chemical Co., Israel) at the lower second molar under anaesthesia. Controls were untreated
- Animals killed by decapitation 7 days later.
- Mandibles were excised, fixed in Zamboni's fixative and decalcified with a 10% EDTA solution for 2 weeks and embedded in paraffin
- The embedded samples were sectioned in the mesio-distal plane parallel to the long axis of the first molar at 15 mm thickness
- The sections were stained with haematoxylin and eosin, toluidin blue, Masson's trichrome stain (for collagen. Osteoclasts were detected using tartrate-resistant acid phosphatase (TRAP) histochemistry according to the procedure described by Katayama et al. (1972).

2. Alveolar bone histomorphometry - Alveolar bone level measurements

- The distance between the level of alveolar bone crest (CA) and the cemento-enamel junction (CEJ) was measured on three random sections at the mesial side of the mandibular second molar in nine animals (Figure 1)
- A total of 27 sections were analyzed
- Mean data for each mandible in each animal were expressed in microns.

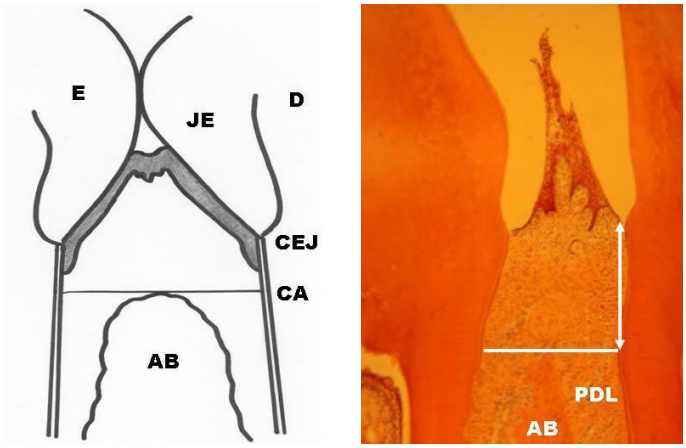



Figure 1. Schematic drawing illustrating gross structures of interdental (mesio-distal cross-section) gingival tissues at rat molars. Arrows show the distances measured histometrically.

3. Alveolar bone histomorphometry - Number of osteoclasts

- The number of on-bone osteoclasts on the mesial and distal surface of the alveolar bone between the first and second mandibular molar were counted
- The mesial surface is defined as that closer to the first molar, while the distal is that closer to the second molar
- The data were expressed as the mean number of cells per mm of bone surface measured

## Results

- The histometric measurements estimating the distance between the CEJ and the level of the alveolar bone crest in random sections at the mesial site of the lower second molars revealed an 80% increase in this measure in LPS-injected animals compared to controls (468±230µm control, 830±27µm LPS, ** p<0.01) at 7 days post-LPS (Figure 2 and 3)

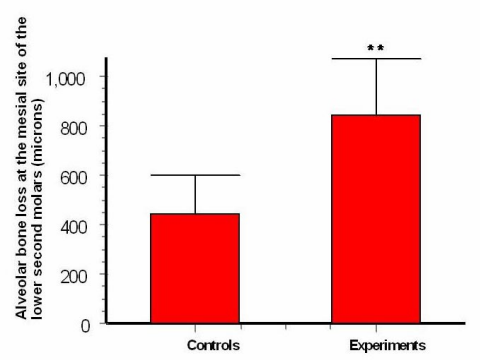



Figure 2. Alveolar bone loss at the mesial site of the lower second molars in controls and experimental animals.

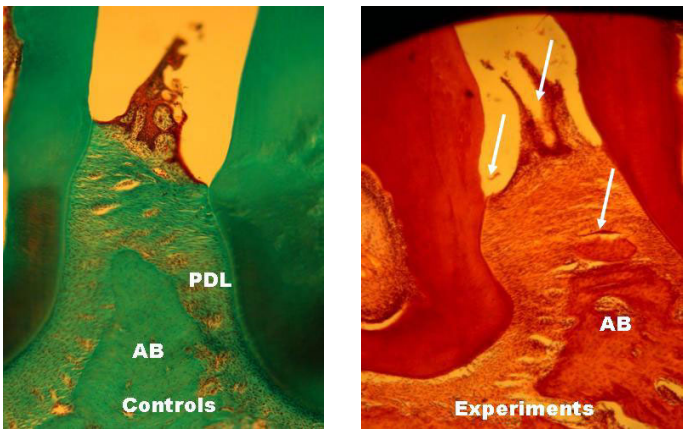
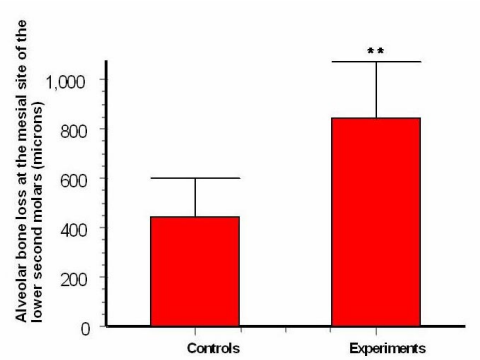



Figure 3. Mesio-distal cross-sections of the interdental gingival tissues between the first and second lower molar. In LPS-induced periodontitis (right picture) the interdental papilla show crater-like lesions, development of periodontal pockets and degradation of the interdental bony septa (arrows) that are not seen in controls (left picture).

- There was no significant difference in numbers of active osteoclasts per mm between control and LPS-injected animals (Figure 4A)
- There was a significant correlation between the number of active osteoclasts and the length of resorbing bone surface (p<0.05) (Figure 4B).



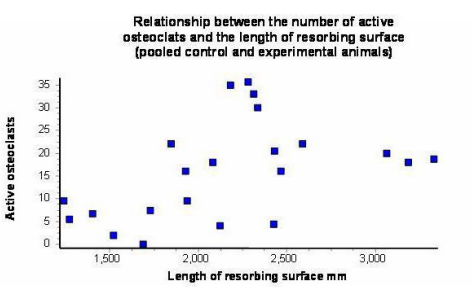



Figure 4. A. Osteoclast number per mm alveolar bone surface of the interdental septum between the first and second molars in controls and experimental animals.

Figure 4. B. Relationship between the number of active (on-bone) osteoclasts and the length of resorbing surface (pooled control and experimental animals).

- Osteoclast number per mm bone length was significantly greater on the mesial than the distal surfaces of alveolar bone in both control ($p = 0.015$) and experimental animals ($p = <0.001$) (Figure 5).

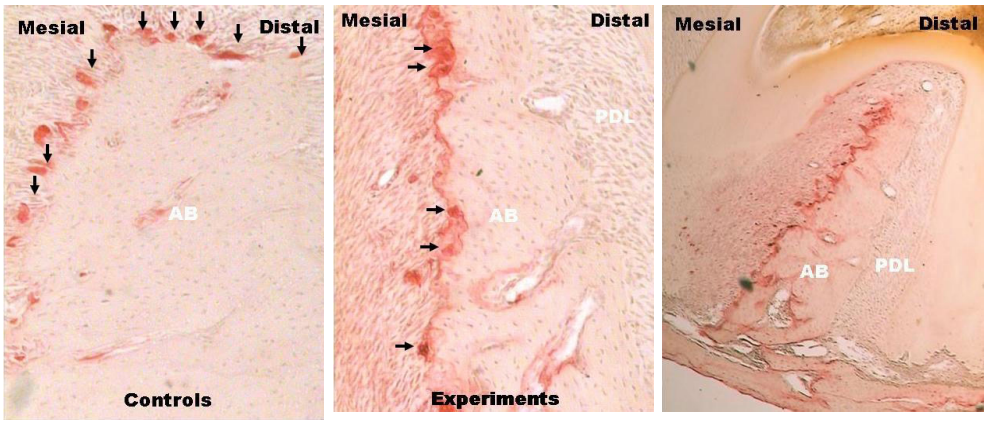



Figure 5. TRAP-positive active osteoclasts can be easily identified in mandibular sections (arrows) from control (left) and experimental animals (right).

More osteoclasts are present on the mesial surface of the alveolar bone

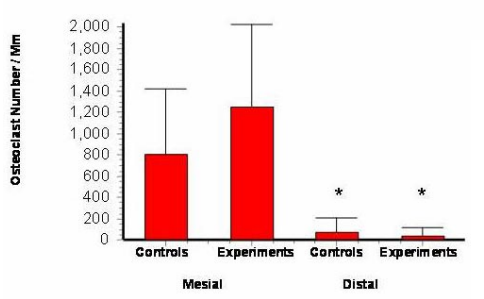



Figure 5C. Osteoclast number per mm bone length on the mesial and distal alveolar bone surfaces in control and experimental animals.

## Conclusions

Periodontal disease has been previously induced in rats using indigenous plaque, experimentally introduced organisms, experimentally introduced bacterial products or by placing a ligature. The clinical and histological findings in experimental periodontal disease in rats are similar to findings in man (Klausen, 1991). In LPS induced experimental periodontitis, a significant increase in osteoclast number is associated with increased alveolar bone loss. This experimental model can be used to investigate the physiological sequelae to alveolar bone loss.

## Literature

Klausen B - Microbiological and immunological aspects of experimental periodontal disease in rats: a review article. Journal of Periodontology 1991, 62, 59-73.
Katayama I, Li CY, Yam LT - Histochemical study of acid phosphatase isoenzyme in leukemic reticuloendotheliosis. Cancer 1972, 29, 157-164.

## Abbreviations

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| AB | alveolar bone |
| CA | alveolar bone crest |
| CEJ | cemento-enamel junction |
| D | dentin |
| E | enamel |
| JE | junctional epithelium |
| LPS | lipoppolysaccharide |
| PDL | periodontal ligament |
| TRAP | tartrate-resistant acid phosphatase |

*This Poster was submitted by Assist. Prof. Dr. Dr. Alexandrina L. Dumitrescu.*

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**Poster Faksimile:**