HISTOLOGICAL COMPARISON OF PERIODONTAL INFLAMMATORY CHANGES IN TWO MODELS OF EXPERIMENTAL PERIODONTITIS IN THE RAT. A PILOT STUDY

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INTRODUCTION

Animal models have been used to evaluate the pathogenesis of periodontal diseases and various periodontal treatment modalities. Human longitudinal studies of periodontal diseases pose many problems such as determining the level of disease activity, individuals at risk and susceptibility to disease progression. It is important to choose a laboratory animal model that has similar characteristics of human anatomy and periodontal disease. Features of periodontal diseases in humans and animals very greatly depending upon which form of the disease is present and the stage of the development. Inflammatory destruction of the periodontium can be spontaneous or experimentally induced in most mammalian species.1

Periodontal disease has been demonstrated to be induced in rats in relation to indigenous plaque, experimentally introduced organisms, bacterial products or by placing a ligature.2
Lipopolysaccharide (LPS) is a cell wall constituent of virtually all subgingival Gram-negative organisms. These molecules are known to induce PMN leukocyte infiltration in inflamed periodontal tissues, oedema and vascular dilatation, absence of osteoblasts, disappearance of osteocytes and to have a stimulatory effect on osteoclasts and on collagen phagocytosis. LPS is a potent inducer of inflammatory cytokines synthesis: IL-1, PGE$_2$, TNFα, IL-6 and IL-8.

Several studies have used LPS to induce periodontal inflammation in rats. Topical application of LPS results in typical inflammatory changes such as junctional epithelial disruption, infiltration of leukocytes and oedema of the subepithelial connective tissue. However use of topical application of LPS may result in more variable changes due to loss of solution into the oral cavity. Injection of LPS into rat or mouse gingiva has been described, both bone loss, and connective tissue breakdown have been reported, while the time course of inflammation and bone loss following a single injection of Salmonella typhimurium in the rat was also evaluated.

The absence of dental plaque, the non typical extent of the tissue destruction (cratering), the absence of infiltrates containing plasma cells and lymphocytes, and the presence of neutrophils and macrophages in areas of active tissue destruction suggest that other factors besides subgingival plaque formation may be of importance in the etiology of periodontal disease in rats. Therefore, it appears that the laboratory rat, although an acceptable model for studying calculus and caries, has limitations as a model for periodontal disease. Periodontal disease in rats is different from that of humans. After inoculation of microorganisms into germ-free rats, periodontal destruction occurs very rapidly, so there is no need for inducing disease with ligatures. The rat is relatively resistant to periodontal disease and is therefore used mostly for oral microflora research. Another difference between the rat and human periodontal disease is that instead of the lesion extending along the root surface as in man, the most apical extent of the lesion is located along the central part of the interdental tissues. Bone loss could occur without apical migration of the junctional epithelium. The gingival response involved is an acute, not a chronic, immune infiltrate.

The aims of the present study were:
1. To histologically compare periodontal inflammatory changes in two models of experimental periodontitis in the rat;
2. To determine the time course of inflammation in these two models.

MATERIALS AND METHODS

All animal experiments were done in accordance with the UK legislation [Animals (Scientific Procedures) Act, 1986]. Male Wister rats (225 - 250g) were anaesthetised with a mixture of fentanyl citrate (0.1 mg/kg) and fluanisone (3 mg/kg) (Hynorm®; Janssen Beerse 0.3 mg/kg) administered intramuscularly, followed by 2.5 mg/kg diazepam administered intraperitoneally.

Periodontitis was produced in two rats by a ligature of 0/0 braided silk placed around the cervix of the upper right second molar. The ligature was knotted on the palatinal and interdental side, so that it remained subgingivally in the vestibular side and supragingivally in the palatinal side. In other two rats the periodontitis was induced by intrasulcular 1 µl injection of Lipopolysaccharide from Salmonella typhimurium (Sigma-Chemical Co., Israel) at the upper right second molar. Five animals were untreated to act as completely uninfammed control animals for comparison. Animals were killed at 2 and 7 days post injection (n = 2 per group) by decapitation under halothane-induced anaesthesia. The animals were sacrificed after 2 and 7 days of periodontitis and the jaws were excised.

Formalin-fixed specimens were decalcified with a 10% EDTA/7.5% polyvinylpyrolidone solution for 2 weeks and embedded in paraffin. Mesiodistal sections from control and inflamed animals stained with hematoxylin and eosin, toluidine blue and collagen staining - Masson's trichrome stain were used to evaluate periodontal conditions at the microscopic level.

RESULTS

Animals showed no obvious signs of systemic illness throughout the period of the study. At 2 days after surgical placement of the silk ligature around the cervix of the second upper molar histological evaluation of the interproximal periodontal tissues did not reveal significant pathological changes compared with the controls. (Fig. 1A,B) Seven days from the induction of periodontitis, an important interradicular alveolar bone loss was observed. (Fig IC) In addition to enhanced neutrophil migration, the gingival response comprises in both the marginal and the interdental regions, an accumulation of inflammatory cells, mainly neutrophils, a few monocytes and small lymphocytes in a narrow zone at the epithelial-connective tissue interface. The histopathological examination of the periodontal structure showed alveolar process...
integrity and lack of neutrophils and osteoclasts in the periodontal tissues of the contralateral maxillary upper molars. (Fig. 1D)

**Figure 1.**

- **A.** Mesiodistal sections illustrating the interdental gingiva in the maxillary molar region of the rat after placing a ligature (2 days observation) (collagen staining - Masson’s trichrome stain).
- **B.** Mesiodistal sections illustrating the interdental and interradicular bony septa in the maxillary molar region of the rat after placing a ligature (2 days observation) (hematoxylin and eosin staining).
- **C.** Mesiodistal sections illustrating the changes in the interdental and interradicular bony septa in the maxillary molar region of the rat after placing a ligature (7 days observation) (hematoxylin and eosin staining).
- **D.** Mesiodistal sections illustrating the changes in the interdental bony septa in the contralateral maxillary molar region of the rat after placing a ligature (7 days observation) (hematoxylin and eosin staining). Bar 5 mm
Figure 2. A. Mesiodistal sections illustrating the changes in the interdental and interradicular bony septa in the maxillary molar region of the rat after LPS injection (2 days observation) (toluidin blue staining); B. Mesiodistal sections illustrating the changes in the interdental gingiva and the interdental bony septa in the maxillary molar region of the rat after LPS injection (7 days observation) (hematoxylin and eosin staining) Bar 5 mm

Compared to the ligated animals, all LPS injected animals showed at least one of the following signs in the interdental area between the maxillary first and second rat molars: crater-like breakdown of the interdental papilla, disruption of transeptal collagen fibres or disruption and apical migration of the junctional epithelium. (Fig. 2A) The number of inflammatory cells (lymphocytes and macrophages) both in the connective tissue and epithelium, was significantly higher in all LPS injected animals compared to the ligature-induced periodontitis. Significant alveolar bone loss was observed beginning at 2 days after periodontitis induction, reaching a maximum at 7 days. (Fig. 2B) The histopathological examination of the LPS injected side showed collagen degradation in the alveolar process, characterized by a dense inflammatory infiltration spread to lamina dura, leading to its evident destruction. The alveolar process damage in the LPS injected side was graded significantly more intense in 7 days than in ligature group at the same time course of inflammation.

Study of active osteoclasts at the interdental septum between the first and second molars showed that there was active bone resorption in control animals as indicated by the presence of these cells on the bone surface. Analysis of the osteoclasts following LPS injection showed a significant increase in the number of active cells present (corrected for the length of bone surface) and an increase in their activity (upward shift in the regression line compared to control data) at the site of injection. This indicates that LPS may have a dual action in stimulating osteoclasts in bone resorption - stimulation of activity in addition to increased number/recruitment.

DISCUSSIONS

Most histological features are similar in rats and humans, except sulcular gingival epithelium that is keratinized. It has been suggested that this keratinization of the oral sulcular epithelium might create a better barrier to bacterial products. However, Romanowski et al. have clearly showed that tracers placed in the sulcular region enters the junctional epithelium rather than the adjacent keratinized tissues, and passes preferentially along the basal lamina or within intercellular spaces closely adjacent to the tooth surface.

There is rapid wear of the occlusal surfaces with continuous eruption of the teeth and apposition of cementum and bone. This causes progressive changes in tooth position, especially the molars, which continuously move in an occlusal-distal-buccal direction. The bony structure of the rat maxilla is altered throughout its relatively short life spans by continuous growth and the eruption and drifting of teeth. One of the more obvious changes of alveolar bone structure is a pronounced narrowing of the interdental bony septum with aging. This loss of
interdental bone width begins early in the live span of the rat and it is believed to result from a difference in the drift rates of adjacent molars. Rat molars are known to drift distally through alveolar bone, and to stimulate both resorptive activity (in advance of each drifting root) and depository activity (behind each drifting root).\textsuperscript{19}

The most frequently used rat strain in periodontitis studies is the Sprague-Dawley strain, but other strains have also been used successfully.\textsuperscript{2}

Periodontal disease have been demonstrated to be induced in rats in relation to indigenous plaque experimentally introduced organisms, experimentally introduced bacterial products and by placing a ligature.\textsuperscript{2,16,25-42} Shojo et al.\textsuperscript{a} has induced experimental periodontitis in rats by placing a standardized elastic ring (diameter: 2.0 mm, thickness: 1.3 mm, width: 0.9 mm) around the cervix of the right mandibular first molar to induce inflammatory periodontitis.\textsuperscript{42}

The left side of the mandible was not treated, and was used as a control side. After 8 days, the presence of an elastic ring around the neck of the mandibular 1st molar induced an acute inflammatory reaction in periodontal tissues: vertical resorption of the bone in the interdental area between the 1\textsuperscript{st} and 2\textsuperscript{nd} molars, widening of the ligamental space around the roots of the 1\textsuperscript{st} and 2\textsuperscript{nd} molars, together with significant bifurcation involvement, were noted.

The clinical and histological findings in experimental periodontal disease in rats are similar to findings in man.\textsuperscript{2} Gingival bleeding was observed on days 3, 7 and 14 after healthy rats were placed in contact with Streptococcus mutans 6715, Actinomyces viscosus ATCC 33277, Fusobacterium nucleatum ATH101, Bacteroides gingivalis ATCC 19246.\textsuperscript{21}

Histologically, after large adherent plaque deposits were present in interdental spaces, migration of the epithelial attachment and an increased incidence of rete peg formation can be commonly observed.\textsuperscript{20}

Many polymorphonuclear leukocytes can be detected in the plaque and on the surface of the gingiva, having presumably migrated through the sulcus.\textsuperscript{25}

An inflammatory cell infiltrate containing T and B lymphocytes, macrophages, and polymorphonuclear leukocytes appears in the connective tissue.\textsuperscript{24,25,43}

Different changes were induced after inoculation with Gram-negative organisms compared with Gram-positive bacteria. No plaque was found at any stage of the experiment when gnotobiotic rats were monoinfected with a Gram-negative, anaerobic rod isolated from a case of human periodontitis.\textsuperscript{6}

Inflammatory reactions, as evidenced by the infiltration of leukocytes, were minimal, nor were more lymphocytes than normal present. No plasma cells were seen, but they are normally rare in rats. The bone destruction was accompanied by numerous osteoclasts, present in considerable greater numbers than normal on the crest and other aspects of the alveolar bone.\textsuperscript{6,26}

After monoinfection with Gram-positive (A. naeslundii) large adherent plaque deposits were present in the interdental spaces. The alveolar bone of these interdental areas invariably demonstrated scalloping of the surface and several small osteoclasts. These small osteoclasts frequently contained no more than two or three nuclei. The osteoclastic activity was discontinuous, short periods of vigorous osteoclastic activity being followed by longer periods of inactivity during which the alveolar crest may be covered with osteoblastic cells or in many cases devoid of bone cells. The net effect in the monoinfected rats is rapid bone loss. Periods of osteoclastic activity were associated with dense inflammatory infiltration of the interdental tissue, ulceration of col epithelium and the presence of large adherent plaques of Actinomyces.\textsuperscript{43}

Assessment of periodontal inflammation in experimental periodontitis in rat can be done by different methods: semi-quantitatively by clinical examination under a dissecting microscope, morphometric measurements of the distance cemento-enamel junction to alveolar bone crest on defleshed jaws, radiographic methods, biochemical methods and histological studies.\textsuperscript{22,25,29-31,35,41,43-46}

The present study showed that at 2 days after surgical placement of the silk ligature around the cervix of the second upper molar histological evaluation of the interproximal periodontal tissues did not revealed significant pathological changes compared with the controls. Only after 7 days after periodontitis induction, an important interradicular alveolar bone loss was observed. Furthermore it was found that LPS injected animals showed significant alveolar bone loss beginning at 2 days after periodontitis induction, reaching a maximum at 7 days.

Since LPS injection resulted after seven days in more periodontal bone loss than did one week ligation, this model may be more advantageous in research studies. It has also the advantage of lower cost, shorter duration and less manipulation of the animals.

**CONCLUSIONS**

In summary, the article presents the histological comparison of two simple reproducible rat model of experimental periodontitis using a ligature or
intrasulcular injection of LPS. Histologically, there were noted many of the features of human periodontitis in the experimental animals, particularly apical migration of the junctional epithelium, collagen breakdown and significant alveolar bone loss. At two and seven days post-injection periodontal inflammatory changes were significantly increased compared to controls. These observations suggest that at one week after LPS injection the inflammation is well established and can be a good reproducible model for study of inflammatory bone loss in the rodent to be also used to investigate the effects of therapeutic agents on control of bone loss.

REFERENCES


