Topical application of tetracycline in regenerative periodontal surgery in beagles

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This study was designed to test the effect of tetracycline on healing subsequent to periodontal surgery. Aqueous solutions of tetracyclines are highly acidic and may therefore represent a suitable substitute for citric acid. Furthermore, tetracyclines react with dental hard tissues to form long-lasting antimicrobial compounds, and they have a retarding effect on pellicle and plaque formation and an antienzymatic effect. The alveolar bone around mandibular premolars was surgically reduced up to 6 mm from the cementoenamel junction in two beagles. The denuded root surfaces were exposed to the oral environment during 3 months without plaque control. Regenerative surgery was then carried out, using root surface conditioning with 1% tetracycline and coronally repositioned flaps. Six months later, histologic evaluation showed connective tissue attachment extending to the cementoenamel junction in most of the specimens. Superficial root resorption was prevalent in the cervical region, below which a collar of replacement resorption partly surrounded the roots in a characteristic manner. Morphometric analysis showed that attachment gain was similar to that obtained with citric acid in a preceding series of seven dogs. These preliminary results indicate that connective tissue attachment gain after topical use of tetracycline is similar to that obtained with citric acid. In addition, the antibacterial capacity and biological effects of tetracycline warrant further study of its possible clinical use in periodontal reconstructive surgery. □ *Experimental study; histopathology; root resorption*

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Current concepts of repair after periodontal regenerative surgery hold that regeneration of a functional attachment apparatus requires the participation of periodontal ligament cells. Fiber attachment will then occur through the formation of new cementum within 3–6 weeks.

Root surface conditioning by citric acid may advance the healing process. The rationale behind this treatment as originally suggested was that demineralization of the planed root surface might stimulate formation of new hard tissue—that is, cementum—on that surface and, thus, ensure attachment of connective tissue fibers (1). Subsequent studies, however, showed that denudation of collagen fibrils in the root dentin or cementum matrix facilitates interdigitation with new collagen fibrils in the healing connective tissue, resulting in the establishment of an attachment mechanism within the 1st week of healing (3).

Whereas this approach repeatedly has led to improved healing results in regenerative surgery of experimentally induced and naturally occurring periodontal defects in dogs (2, 4–7) and monkeys (8, 9), healing improvement has been less dramatic in humans, possibly owing to insufficient flap coverage, failure to remove all bacterial contaminants and postoperative infection (10–16).

More recently, interest has been focused on the potential of tetracyclines for conditioning of the root surface in studies of new attachment. In addition to their immediate antibiotic effect, topically applied tetracyclines react with dental hard tissues, from

Table 1. Experimental material

Dog 1	P_4	P_3	P_2	P_2	P_3	P_4
Dog 2	P_4	P_3^*	P_2	P_2^{**}	P_3^*	P_4

* Tooth lost in surgery.
** Surgical failure.

which they are slowly released in an antibiotically active form (17–21), and they have a retarding effect on pellicle and plaque formation (22, 23) and antienzymatic effects that may retard collagen breakdown and reduce inflammatory reactions (24, 25). Tetracycline conditioning of dentin may promote fibroblast adhesion and growth (26). These observations have led us to examine the potential of tetracycline as a substitute for citric acid in regenerative flap surgery, using our established model in beagle premolars.

Materials and methods

Animals

Two beagles, approximately 2 years old, were used for the study. The mandibular right and left second, third, and fourth premolars (P_2, P_3, and P_4) were subjected to experimentation (Table 1).

Periodontal defect creation

Horizontal circumferential periodontal defects were created by removing alveolar bone around the experimental teeth as described previously (2).

Regenerative surgery

Surgical procedures and postoperative care followed the protocol described previously (2) except that after root planing, instead of citric acid, a 1% aqueous solution of tetracycline HCl (pH 2.6) was applied to the root surfaces for 5 min, after which the wound was rinsed with isotonic saline.

The length of denuded root surface appeared to have increased by approximately 1 mm during the 3-month plaque accumulation period (Table 2). Because of the extreme size of the defects and consequent mobility of the teeth, 2 premolars were lost during surgery, leaving 10 teeth for observation. Among these, one tooth was clinically diagnosed as an overt surgical failure the 1st day postoperatively, apparently a result of early suture failure.

Histologic observation

The dogs were killed 6 months after regenerative surgery, and specimen blocks consisting of tooth, alveolar bone, and periodontal soft tissues were processed for histologic examination as described previously (2). Serial, longitudinal 7-μm sections were cut in the buccolingual plane. For histometric analysis, two sections were chosen from each tooth, one representing the largest buccolingual dimension of the mesial root and one from the corresponding area of the distal root.

The following measurements were made from the buccal and lingual aspects of these roots (four surfaces per root):

(a) Root denudation: the distance from the cementoenamel junction (CEJ) to the apical extent of the root planing;

(b) Epithelial downgrowth: the distance

Table 2. Root denudation by tooth position and root surface

Surface	Clinical measurement at defect creation (mm)	Clinical estimate at surgery (mm)	Histometric analysis (mm)
All P_2 surfaces	~4	~5	4.9 ± 1.0
All P_3 surfaces	~5	~6	5.5 ± 1.6
All P_4 surfaces	~6	~7	7.0 ± 1.9

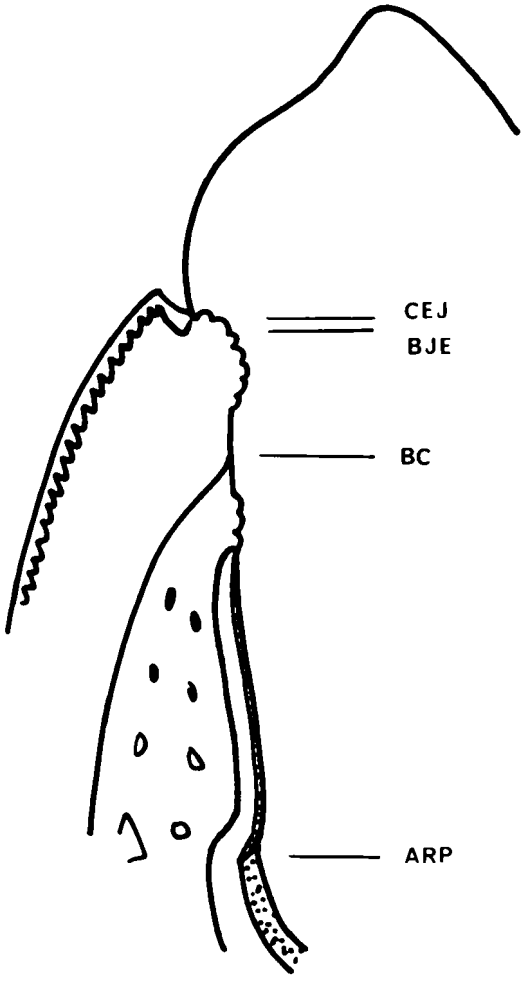

Fig. 1. Schematic illustration of characteristic healing pattern. CEJ = cementoenamel junction; BJE = base of junctional epithelium; BC = bone crest; ARP =apical extent of root planing.

from the CEJ to the apical extension of the junctional epithelium;

(c) Connective tissue attachment: distance *b* subtracted from distance *a*;

(d) Bone apposition: the distance from the most coronal alveolar bone crest to the apical extent of the root planing;

(e) Root resorption: the total vertical length along the root surface showing distinct concavities caused by resorption activity (minor surface irregularities excluded);

(f) Ankylosis: total vertical length along the root surface showing ankylosis of alveolar bone to the root surface.

The healing results using tetracycline conditioning were compared with those obtained in a preceding study using citric acid (2).

Results

Of the 12 teeth originally designated for the study, 9 teeth were available for histologic observation and histometric analysis of the healing result.

In all cases, coronal regeneration of connective tissue attachment to the root surface had occurred practically to the CEJ. Two of the 36 root surface areas examined showed an uncomplicated regeneration of periodontal ligament and alveolar bone coronal to the apical extent of root denudation and an absence of ankylosis and/or root resorption.

In most of the surfaces, however, healing followed a characteristic pattern, as shown diagrammatically in Fig. 1. An area of relatively deep resorption was generally seen immediately apical to CEJ. In many instances, repair was occurring, and the resorption defects showed a lining of cellular cementum. In other specimens these defects appeared to be actively progressing at the 6-month observation time. Apical to the cervical resorption, an area of ankylosis was present in most specimens. In some instances ankylosis had been preceded by superficial root resorption. Characteristic examples of healing are shown in Figs. 2 and 3.

Examination of step-serial sections indicated that, typically, the ankylotic process formed a collar around each tooth. Such examination also showed that ankylosis was prevalent in the fornix of the furcations.

Apical to the area of ankylosis the root-planed surfaces showed an absence of resorption and were surrounded by an inflammation-free periodontal ligament and new alveolar bone.

In no instance did resorptions extend to the pulp. The periodontal tissues apical to the extent of root planing also appeared normal.

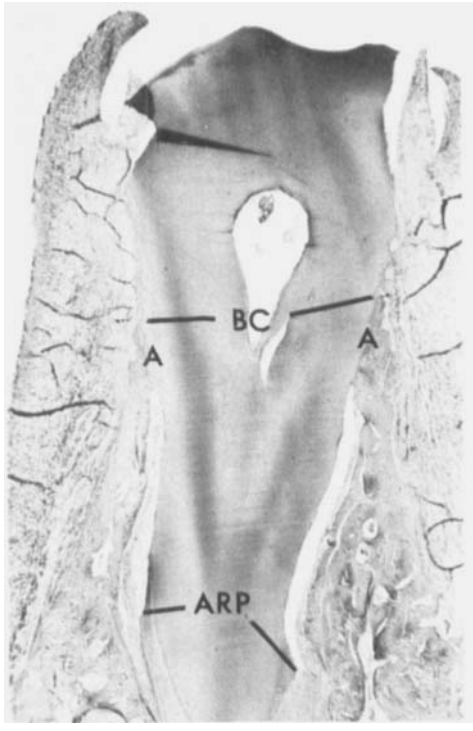

Fig. 2. Photomicrograph of vertical midroot section, illustrating regeneration of connective tissue and alveolar bone. Coronal to the apical extent of root planing (ARP) there is 3–3½ mm of periodontal ligament regeneration and a 1-mm-wide collar of ankylosis (A). Gingival connective tissue attachment extends coronally to the bone crest (BC). (Magnification, ×10.)

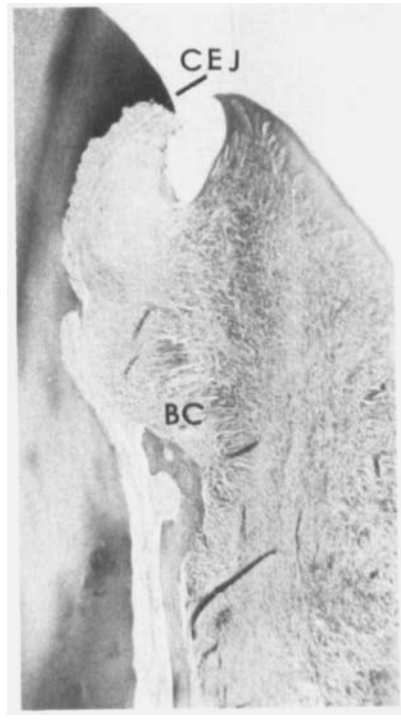

Fig. 3. Connective tissue new attachment extending to the cementoenamel junction (CEJ) and absence of ankylosis. Coronal to the bone crest (BC), connective tissue fibers are attached to a relatively deep resorption defect. (Photomicrograph; magnification, ×25.)

The results of the histometric analysis are presented in Tables 2 and 3.

Discussion

With the exception of one overt clinical failure, regenerative surgery in this study resulted in complete coverage of all denuded root surfaces by connective tissues. The extent of alveolar bone regeneration averaged 62% of the length of the root-planed surfaces (range, 26–94%). However, ankylosis had in most instances occurred in a limited area at the most coronal extent of bone regeneration. Moreover, root resorption was a complicating healing response that showed a regular and characteristic distribution immediately below the CEJ.

These healing results should be compared with those reported in a preceding study (2). Both studies followed the same experimental protocol, and the surgical procedures were carried out by the same operators. The only experimental variable was that, in the present study, root conditioning was done with 1% tetracycline instead of saturated citric acid. In both studies coronal regeneration of connective tissue attachment generally extended to the CEJ. When citric acid was used, however, extensive root resorption occurred, with the consequence that 22 of 120 root surfaces had to be excluded from morphometric analysis (2). In the present study, albeit with a limited number of specimens, resorption defects were generally limited in size and never obscured the identification of root surface landmarks. Although direct comparisons are not possible, it seems that root conditioning with tetracycline com-

Table 3. Histometric analysis of 36 root surfaces 6 months after regenerative periodontal surgery with tetracycline conditioning

	Mean ± SD (mm)	Range (mm)
Root denudation	5.9 ± 1.8	3.1–9.9
Epithelial downgrowth	0.0 ± 0.1	0.0–0.7
Connective tissue attachment	5.9 ± 1.8	3.1–9.9
Bone regeneration	3.6 ± 1.3	1.8–6.6
Root resorption	1.5 ± 1.0	0.0–4.0
Ankylosis	0.9 ± 0.8	0.0–2.6

pared with citric acid may have produced a more favorable, although not ideal, healing result.

This study once more documents the biologic potential that exists for regeneration of connective tissue attachment to root surfaces that have been involved in periodontal disease and have been denuded of their original periodontal support to the extent that they show extreme mobility. As frequently discussed, the healing reaction in the cervical region may be dependent on the origin and nature of those tissue elements from which the regenerated tissues originate—that is, periodontal ligament, alveolar bone, or gingival connective tissue (27–33). It was interesting that on 7 of 36 surfaces the alveolar bone had regenerated to within 1 mm from the CEJ, and these were not among those surfaces showing the most extensive ankylosis and resorption.

On the basis of our early studies with the same experimental model, it can be assumed that very limited regeneration of connective tissue attachment would have occurred if root surface demineralization had not been used. Apparently, the more superficial demineralization obtained with tetracycline (about 1 μm (unpublished results)) than with citric acid (3–5 μm (3)) was sufficient to achieve the necessary exposure of matrix collagen.

The results of the present study must be interpreted with caution owing to the limited size of the experimental material and the fact that experiments were carried out on animals in which periodontal disease with advanced lesions had been artificially induced only 3 months before regenerative surgery. However, the healing results were in no respect inferior to those achieved with citric acid. Considering the problems of contamination of the wound area which prevail in clinical practice, further studies should be pursued to clarify the benefits of tetracycline as an adjunct in periodontal regenerative surgery under clinical conditions.

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