Effects of citric acid and fibronectin and laminin application in treating periodontitis


Abstract. To determine the effect on new connective tissue attachment of citric acid conditioning and fibronectin-laminin application in treating naturally occurring periodontitis, all 4 quadrants in each of 2 Beagle dogs were used. Each quadrant included: P1, P2, P3, and M teeth. 2 treatment modalities were employed and comparatively analyzed for differences in histological healing responses at 120 days after surgery. The treatments were: (1) surgery (mucoperiosteal flaps) plus citric acid; (2) surgery plus citric acid followed by fibronectin-laminin application. After scaling and root planing, coronal and root surface reference notches were placed for histometric measurements. Following each of the randomly assigned treatments, flaps were sutured. After sacrifice, tissue blocks of treated areas were decalcified and serially cut, obtaining bucco-lingual and mesio-distal sections. Using a Filar micrometer, 5 distances were measured on the buccal aspect: (1) from root surface notch to alveolar bone crest; (2) from root surface notch to corona! extent of the cementum; (3) from root surface notch to apical extent of the junctional epithelium; (4) from free gingival margin to apical extent of junctional epithelium; (5) from the coronal notch to the alveolar bone crest. Results showed no differences among the 5 measurements between the 2 treatments tested. On mesio-distal sections, surface area determinations were made in the furcations, evaluating the space occupied by new connective tissue, with or without bone, or by epithelium. For this, images were digitized using a Zeiss IBAS Image analysis system with a 4mB of array processor memory coupled to a Newvicon TV camera and a microcomputer. Significant differences were found, with increased values for both regenerative connective tissue and bone when surgery plus citric acid was followed by fibronectin-laminin application. Often, these tissues filled completely furcation areas above root surface reference notches.

Key words: Citric acid - fibronectin - laminin. Accepted for publication 10 August 1986

Cells utilize high molecular weight glycoproteins to attach to the surrounding structures. Fibronectin is a glycoprotein intimately associated with the attachment of fibroblasts to collagen, various cells and their surrounding matrix (Kleinman, Klebe, Martin 1981). Laminin, on the other hand, is utilized by the epithelial cells for adhesion to different substrates and is a significant constituent of basement membranes (Terranova, Rohrbach & Martin 1980).

Recently, the role that attachment proteins may play in new attachment procedures has received considerable attention (Terranova & Martin 1981, 1982). In a previous communication, improvement in new connective tissue attachment to root surfaces of Beagle dogs affected with natural periodontitis occurred when the teeth were surgically treated using a modified Widman flap approach; the roots were demineralized and fibronectin was applied on the inner flap surface and on the roots (Caffesse et al. 1985).

Since laminin is used by the epithelial cells to bind to their substrate, it could be theorized that if laminin were applied to the cervical area of the root, close to the cementoenamel junction, it might enhance the adhesion of the epithelial cells to the tooth in that area, thus delaying their apical migration along the root surface.

The purpose of the present study was to evaluate the use of a fibronectin and laminin combination in the treatment of naturally occurring periodontitis in Beagle dogs.

Material and Methods

2 6-year old female Beagle dogs, with intact dentition were selected from a closed Beagle colony at Laboratory Research Enterprises, Inc. in Kalamazoo, Michigan. Periodontal disease had re-
2 quadrants per dog were treated surgically using a modified Widman flap procedure (Ramfjord & Nissle 1974). After surgical exposure, the roots were thoroughly scaled and root planed, and then conditioned with a sterile solution of citric acid with a pH of 1.0, applied with cotton pledgets for 3 min. The area was then thoroughly rinsed with sterile saline. Repositioning of the flaps at or just coronal to the cemento-enamel junctions was achieved with interproximal interrupted 0000 braided black silk sutures. Suspensory sutures were also used in some cases. The other 2 quadrants were treated similarly, but after the application of citric acid and rinsing with sterile saline, fibronectin and laminin were applied in the following manner. The area was isolated with sterile gauze to prevent contamination with saliva. Fibronectin was then applied with a tuberculin syringe, bathing both the roots and the inner aspect of the flap. Immediately after, laminin was applied by painting the glycoprotein to the cemento-enamel junction areas and the most coronal part of the roots with a brush. Good flap adaptation was achieved by pressing the tissues against the roots and bone. Suturing was subsequently carried out. Fibronectin, of human origin, was purchased from Collaborative Research Inc., Waltham, Massachusetts. 1 ml of sterile saline was added to the vial containing the 1.0 mg of the lyophilized glycoprotein 30 min prior to its use. Laminin, extracted from the EHS tumor grown in mice was provided by Dr. Victor Terranova from the NIDR. It was kept frozen until needed, at which time it was allowed to thaw at room temperature. The concentration was 2.5 mg/ml. Each glycoprotein was applied at the amount of 1.0 ml/quadrant.

During surgery, 1 coronal notch and 1 radicular notch at the level of the alveolar crest, were made to be used as landmarks for histometric evaluation.

After surgery, daily brushing and weekly rubber cup prophylaxis were provided. 120 days later, the animals were sacrificed. The jaws were dissected, fixed, trimmed, decalcified in EDTA, washed, dehydrated, embedded in paraffin and sectioned at 5-6 intervals. Within each quadrant, alternate blocks were designated for either buccal-lingual, or mesial-distal sectioning, involving the furcation areas. The sections were stained with hematoxylin and eosin. Under a binocular light microscope, using a Filar micrometer eyepiece* held parallel to the specimen surface (Nezwek et al. 1980), the following histometric measurements were recorded on the buccal-lingual sections.

(1) From the root surface notch to the alveolar bone crest.
(2) From the root surface notch to
Operated areas healed uneventfully, but they all showed significant gingival recession.

On the buccal-lingual sections, both areas, those treated with citric acid (Fig. 3) and those treated with citric acid, fibronectin and laminin (Fig. 4), showed similar healing. New connective tissue attachment was evident, including cementum deposition in the area of the notch, and some bone regeneration (Fig. 4a). However, a long junctional epithelium also developed, reaching in most instances the coronal aspect of the radicular notch (Fig. 4b). Some areas treated with fibronectin/laminin combination showed ankylosis. However, most of the ankylosed areas were located apical to the radicular notch (Fig. 5).

When the linear measurements recorded were analyzed, no significant differences between procedures were found (Table 1). However, both techniques gained some connective tissue attachment.

On the mesial-distal sections, the furcation areas were evaluated. The areas treated with citric acid only showed
Fig. 4a. Higher magnification of Fig. 4. New connective tissue attachment is evident at the apical 2/3 of the notch, with new cementum (arrows) and bone formation. H&E x100.

Fig. 4b. Higher magnification of the coronal part of the notch seen in Fig. 4. An extension of the junctional epithelium is evident (arrows). H&E x100.

Fig. 5. Buccal-lingual section treated with citric acid and fibronectin/laminin combination. Ankylosis is seen at the middle 1/3 of the root. H&E x10.

more gingival recession than those treated with fibronectin-laminin. Connective tissue proliferated to the coronal level of the notches, and fiber attachment was evident with cementum deposition within the notch areas, and minimal bone growth was seen (Fig. 6). The areas which were treated with fibronectin-laminin combination depicted greater closure of the furcations (Fig. 7), with areas showing complete obliteration (Fig. 8). New connective tissue attachment, cementum deposition and bone growth were evident, with some teeth also showing different degrees of ankylosis.

The areas of the furcation filled with regenerated epithelium were similar for both procedures. However, the areas occupied with new connective tissue, including bone, demonstrated a significantly better response in the areas treated with fibronectin and laminin (Table 2).

Discussion

The present results confirm previous findings in the Beagle dog model relative to the effect of root conditioning with citric acid in new attachment procedures in natural periodontitis (Bogle et al. 1981). As reported earlier, those results could be improved by the application of fibronectin under the flap, bathing the gingival connective tissue and the demineralized roots (Caffesse et al. 1985).

The findings from the buccal-lingual sections showed that when laminin was used in addition to citric acid and fibronectin, there were no improvements of results beyond those obtained with the use of citric acid alone, in spite of the fact that fibronectin was also applied.

Although laminin was applied with a paintbrush to the cementoenamel junction area, it was impossible to prevent its apical seepage under the flap into the area previously covered with fibronectin. As a consequence, the result was a long junctional epithelium reaching the coronal border of the notch. It should be remembered that laminin was applied after fibronectin. It would appear that the application of laminin negated the beneficial effects of fibronectin in promoting new connective tissue attachment.

In the furcation areas, however, the findings were different. The distance from the cementoenamel junction to the furcation, and the protection that this
Fig. 5a. Higher magnification of the middle 1/3 of the root showing ankylosis (arrows). H&E ×25.

Fig. 6. Furcation area treated with citric acid. Connective tissue growth is evident in the area of the notches. However, no bone growth has taken place in this instance. H&E ×10.

Fig. 7. Furcation area treated with citric acid and fibronectin/laminin combination. Epithelium, connective tissue and bone are seen coronal to the notches. The furcation is partially filled. Areas of ankylosis (black arrows) and arrested resorption (white arrows) are seen. H&E ×25.

Anatomical feature provided per se, made it improbable that laminin could leak deep enough so as to involve the entire furcation areas. Furthermore, it should be kept in mind that in spite of the gingival recession produced, the flaps were sutured at the cementoenamel junction away from the furcation areas. The degree of epithelial fill found in the furcation was similar with both treatments, which would indicate that this area was not affected by the applied laminin. This could be interpreted as due to the effect of the fibronectin application in areas where the laminin, applied afterwards, did not have the possibility to reach. Complete obliteration of some furcation areas was seen, with connective tissue attachment to new cementum, bone fill and even ankylosis areas. Most of the ankylosis was located apically to the notches; hence, it is difficult to conclude whether the treatment approach was responsible for these changes. However, reports indicate concern about the development of ankylosis after root conditioning (Magnusson et al. 1984). As shown by Karring et al. (1980) and Nyman et al. (1980), the source of cell repopulation during healing may be the factor determining the production of ankylosis.

It is evident that the intention of halting the apical migration of the junctional epithelium during healing by applying topical laminin on the cervical area of the root is not supported by the present findings. Furthermore, evidence seems to indicate that laminin may have a much more significant role in the pathogenesis of disease than in treatment (Boekeeloo et al. 1986) However, the effect on healing of laminin alone, or with citric acid but without fibronectin, should be investigated.

Conclusion

Within the limits of the present investigation, it can be concluded that the use of a combination of fibronectin and laminin in new attachment procedures does not seem to be justified.

Zusammenfassung

Die Effekte der Zitronensaure sowie der Fibronectin und Laminin Applikation bei der Behandlung der Parodontitis

Zur Bestimmung des Effektes der Imprägnierung mit Zitronensaure, wie auch der Fibronectin-Lamininapplikation auf die Neubildung bindegeweblichen Attachments bei der Behandlung natürlich vorkommender Paro-
Table 1. Mean linear measurements (mm) on buccal-lingual sections; 2 sample t-tests

<table>
<thead>
<tr>
<th></th>
<th>Citric acid</th>
<th>Fibronectin</th>
<th>Difference</th>
<th>Standard deviations</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>notch to bone</td>
<td>0.220</td>
<td>0.160</td>
<td>0.060</td>
<td>0.133</td>
<td>N.S.</td>
</tr>
<tr>
<td>notch to cementum</td>
<td>3.082</td>
<td>2.465</td>
<td>0.617</td>
<td>2.08</td>
<td>N.S.</td>
</tr>
<tr>
<td>notch to bottom junctional epithelium</td>
<td>4.220</td>
<td>3.430</td>
<td>0.790</td>
<td>2.23</td>
<td>N.S.</td>
</tr>
<tr>
<td>gingival margin to bottom junctional epithelium</td>
<td>4.167</td>
<td>4.160</td>
<td>0.007</td>
<td>0.470</td>
<td>N.S.</td>
</tr>
<tr>
<td>coronal notch to bone</td>
<td>17.105</td>
<td>14.845</td>
<td>2.260</td>
<td>3.855</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Résumé

Effets de l'acide citrique et de l'application de fibronectine et de laminine dans le traitement de la parodontite

La réalisation d'une nouvelle attache conjonctive suite à l'utilisation d'acide citrique et l'application de fibronectine-laminine dans le traitement de la parodontite naturelle a été étudiée au niveau des quatre quadrants de deux chiens briquet. Chaque quadrant contenait les dents P3, P4, P5 et M1. La guérison histologique a été comparée 120 jours après la chirurgie utilisant deux modalités de traitement. Ces traitements étaient: 1) chirurgie (lambeaux mucoperiostes) avec acide citrique et 2) chirurgie avec acide citrique puis application de fibronectine-laminine. Après détartrage et lissage radiculaire, des encoches coronaires et radiculaires de référence ont été taillées pour les mesures histométriques ultérieures. A la suite de ces traitements, des lambeaux ont été suturés. Après sacrifice, des biopsies complètes des zones traitées ont été décalcifiées et coupées en série pour obtenir des coupes vestibulo-hanguales et mesio-distales. En utilisant un micromètre filaire, plusieurs mesures ont été effectuées en vestibulaire et en interradiculaire: (1) de l'encoche radiculaire à la crête osseuse alvéolaire; (2) de l'encoche radiculaire à la limite coronale du cément; (3) de l'encoche radiculaire à la limite apicale de l'epithélium de jonction; (4) de la gencive marginale à la limite apicale de l'epithélium de jonction; (5) de l'encoche coronale à la crête osseuse alvéolaire. Les résultats n'ont montré aucune différence quant à ces cinq mesures entre les deux traitements testés. Sur les coupes mesio-distales, l'estimation des différentes surfaces a été effectuée dans les furcations, évaluant l'espace occupé par le tissu conjonctif, avec ou sans os, ou par l'épithélium. Ces images ont été chiffrées avec un système d'analyse d'image IBAS Zeiss avec mémoire, camera et microordinateur. Des différences significatives ont été trouvées avec augmentation des valeurs pour le nouveau tissu conjonctif et l'os lorsque la chirurgie avec acide citrique était suivie d'une application de fibronectine-laminine. Souvent ces tissus remplissaient complètement les zones de la furcation au-dessus des encoches radiculaires de référence.

Références


Table 2. Mean surface area determinations (mm²) of furcation areas; 2-sample t-tests

<table>
<thead>
<tr>
<th></th>
<th>Citric acid</th>
<th>Fibronectin laminin</th>
<th>Difference</th>
<th>Standard deviations</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>intraradicular space</td>
<td>13.035</td>
<td>12.087</td>
<td>0.948</td>
<td>2.48</td>
<td>N.S.</td>
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<td>epithelium</td>
<td>1.722</td>
<td>1.345</td>
<td>0.377</td>
<td>0.51</td>
<td>N.S.</td>
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<tr>
<td>connective tissue</td>
<td>4.597</td>
<td>7.457</td>
<td>2.860</td>
<td>1.28</td>
<td>0.01</td>
</tr>
<tr>
<td>connective tissue plus bone</td>
<td>5.182</td>
<td>8.137</td>
<td>2.955</td>
<td>1.48</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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