Changes of the basal lamina in the rat gingiva receiving incisional wound and bacterial protease application

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Abstract: The purpose of the present ultrastructural study was first to investigate the changes of the basal lamina in the gingiva which were produced by both wounding and bacterial protease application and second to investigate the fate of the changed basal lamina with time. In all 4 groups, animals received an incision in the lingual gingiva. Immediately after incision, wounds of Groups A and C were treated with distilled water, and those of Groups B and D with bacterial protease. Histologically, in Groups A and B on Day 4, the gingival connective tissues showed granulation. In the former group the basement membrane appeared relatively clear, whereas in the latter it was indistinct. In both groups on Day 28, the gingiva appeared normal. Ultrastructurally, in Group C on Day 4, the basal lamina exhibited various changes such as detachment, breaking, thickening, fragmentation and multiplication, but on Day 28, these changes had improved. In Group D on Day 4, the above-mentioned changes were more conspicuous than those of Group C on Day 4, and in the connective tissue, fibroblast-like cells were sometimes in contact with the basal cell. On Day 28, the changes of the basal lamina had improved, but they were more marked than those of Group C on Day 28. In conclusion, the present results suggested that the rat gingiva receiving wounds and bacterial protease showed conspicuous changes of the basal lamina and that these changes were reduced in intensity with the passage of time.

Introduction

The gingiva consists of both the epithelium and the underlying connective tissue. Histologically, the gingival epithelium is separated from the underlying connective tissue by the basement membrane and ultrastructurally they are separated from each other by a thin layer of specialized extracellular material called the basal lamina. The basal lamina is considered to have important roles in cell proliferation, migration, and differentiation. It was reported that changes of the basal lamina occurred in the gingiva with wounds or inflammation. However, information regarding the fate of the changed basal lamina with the passage of time was not available in the literature. Gingival wounds are common, some are sustained accidentally during mastication of food and others are inflicted in the course of dental treatments. Bacterial protease, one of several enzymes elaborated by dental plaque, has been considered a causative factor in gingival inflammation. The purpose of the present ultrastructural study was first to inves-
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basal lamina with the passage of time.

Material and Methods

Four groups (Groups A-D) of Wistar rats, 10 animals each, were used in the present study. In the animals of all groups, a frontal incision, 1 mm in depth, was made in the lingual gingiva of the lower incisors with a scalpel. Immediately after incision, the gingival wounds in the animals of Groups A and B received applications of 0.03 ml of distilled water and the same volume of 5% bacterial protease solution (280 units of bacterial protease, Sigma type XIV, /1 ml distilled water) for 1 minute using a small amount of cotton, respectively. The animals of Groups A and B were killed 4 and 28 days after application. The tissues of the gingival wounds were removed, prefixed for 2 hours in a mixed solution of 2 % paraformaldehyde and 2.5% glutaraldehyde in 0.1 molar phosphate buffer at pH 7.4. After a buffer rinse, they were postfixed for 2 hours in 1% buffered osmium tetroxide. The tissues were dehydrated through a graded series of ethanol and embedded in Epon 812. Thin sections were stained with both uranyl acetate and lead citrate and examined ultrastructurally.

Results

Histological observation

In Group A on Day 4, the wound was completely covered by the keratinized epithelium, but some granulation tissue was found

4 μm in a frontal plane. The tissues were examined histologically. The gingival wounds in Groups C and D received the same treatment as those in Groups A and B, respectively. The animals of Groups C and D were killed 4 and 28 days after application. The tissues of the gingival wounds were removed, prefixed for 2 hours in a mixed solution of 2 % paraformaldehyde and 2.5% glutaraldehyde in 0.1 molar phosphate buffer at pH 7.4. After a buffer rinse, they were postfixed for 2 hours in 1% buffered osmium tetroxide. The tissues were dehydrated through a graded series of ethanol and embedded in Epon 812. Thin sections were stained with both uranyl acetate and lead citrate and examined ultrastructurally.

Results

Histological observation

In Group A on Day 4, the wound was completely covered by the keratinized epithelium, but some granulation tissue was found
in the underlying connective tissue (Fig. 1). The basement membrane between the epithelium and the underlying granulation tissue was relatively clear. On Day 28, granulation tissue was not observed below the keratinized epithelium, the basement membrane and the connective tissue appeared normal.

In Group B on Day 4, the wound was also completely covered by the keratinized epithelium (Fig. 2). However, a granulation tissue was present in the underlying connective tissue and it was more marked than that in Group A (Fig. 1). The basement membrane between the epithelium and the granulation tissue was indistinct. On Day 28, as in Group A, a granulation tissue was not found under the keratinized epithelium and the basement membrane and connective tissue appeared normal. 

Ultrastructural observation

In Group C on Day 4, in the junctional area between the epithelium and the underlying granulation tissue, a layer of normal basal lamina was seen just below the basal surface of the basal cell (Fig. 3). In many connective tissue areas, however, detachment, thickening, fragmentation and multiplication of the basal lamina were also observed (Fig. 3). Many anchoring fibrils were attached to one or both sides of these basal laminae. Sometimes, the connective tissue cells, probably fibroblasts, were closely located to the basal cell. On Day 28, a normal basal lamina was seen just below the basal surface of the basal cell (Fig. 4). But the changes of the basal lamina as seen on Day 4 were also observed in the underlying connective tissue area, although they decreased in intensity and amount (Fig. 4).

In Group D on Day 4, in the epithelial-connective tissue junctional area, a normal basal lamina which was usually located just below the basal surface of the basal cell could scarcely be seen (Fig. 5, 6). Detachment, breaking, thickening, fragmentation and multiplication of the basal lamina were often found in
Fig. 5 Group D on Day 4. Normal basal lamina which is usually located just below the basal surface of the basal cell can scarcely be seen. Detachment, breaking, thickening, fragmentation, and multiplication of the basal lamina are found in the underlying connective tissue. (BC) : Basal Cell, (BL) : Basal Lamina, (AF) : Anchoring Fibril ×10,000.

Fig. 6 Group D on Day 4. Normal basal lamina can scarcely be seen. In the deep portion of the connective tissue, detachment, breaking, thickening, fragmentation, and multiplication of the basal lamina are observed. (BC) : Basal Cell, (BL) : Basal Lamina ×5,000.

The present study demonstrated histologically that the area of the incisional gingival wound treated with bacterial protease in Group B on Day 4 had both an indistinct basement membrane as compared with the same area in Group A on Day 4 which had displayed a relatively clear basement membrane and more marked granulation tissue than that treated with distilled water in Group A. The incision areas in both groups on Day 28 exhibited no granulation tissue and had normal basement membranes. These findings may indicate that bacterial protease brought about the destruction of the gingival connective tissue leading to the indistinctness of the basement membrane and a marked production of granulation tissue.

So far, little information concerning the ef-
Effects of bacterial protease on the gingiva in animals or humans has been published. Suzumura et al., studying the effect of bacterial protease on the periodontal tissues in rats histologically, reported that application of bacterial protease induced a marked apical proliferation of the junctional epithelium along the tooth surface. With respect to the effects of bacterial protease on other tissues, there have been a few investigations. Kamata et al. reported that serratell protease caused vascular permeability enhancement followed by edema formation when injected into guinea pig peripheral corneas and subconjunctival space or skin. Kamata et al., studying a possible cause and the difference in clinical severity of serratell keratitis in humans, reported that protease produced by the bacteria was a major destructive factor of the corneal tissue and that protease induced remarkable intrastromal edema and liquefactive necrosis of the corneas.

Ultrastructurally, in the present study, the incisional wounds of the gingiva treated with distilled water in Group C on Day 4 and Day 28 and those treated with bacterial protease in Group D on the same days showed changes of the basal lamina such as detachment, breaking, thickening, fragmentation, and multiplication. However, these changes of the basal lamina were more remarkable in Group D than in Group C. These findings may indicate that application of bacterial protease to the gingival wound enhanced the structural changes of the basal lamina. This enhancement may be related to the degradation of the basal lamina and its surrounding matrix by the action of bacterial protease.

Changes of the basal lamina in the gingiva have been also reported by Takarada et al. who studied ultrastructurally chronic periodontitis in humans. They stated that four mechanisms were considered as causes producing changes of the basal lamina. First, thickening of the basal lamina occurs. Second, detachment of the basal lamina from the basal cell occurs. Third, reproduction of the basal lamina occurs on the epithelial side of the de-

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Fig. 7 This is a higher magnification of part of Fig. 6. Many anchoring fibrils attach to one or both sides of the irregular basal laminae. (BL): Basal Lamina, (AF): Anchoring Fibril ×16,000.

Fig. 8 Group D on Day 4. Normal basal lamina can scarcely be seen. In the underlying connective tissue, the connective tissue cells, probably fibroblasts, are located near the basal cell. (BC): Basal Cell, (CC): Connective Tissue Cell ×16,000.
Fig. 9 Group D on Day 4. Normal basal lamina is not observed. In the underlying connective tissue, the connective tissue cell, probably fibroblast, is touching the basal cell. (BC) : Basal Cell, (CC) : Connective Tissue Cell ×16,000.

detached basal lamina. Fourth, the formation of connective tissue, such as collagen fibers just beneath the newly formed basal lamina, forces the detached basal lamina deeper into the connective tissue. Sugimoto et al.10), studying ultrastructural changes of the basal lamina and anchoring fibrils of human gingiva in vitro, proposed the following process on the mechanisms of changes of the basal lamina in the gingiva. Primary detachment occurs as a sequel to sporadic losses of adhering materials (hemidesmosomes, tonofibrils, and ground substances). Reduplication of basal lamina is produced by basal cells in areas of primary detachment. Secondary complete detachment occurs because of severe loss of adhering materials and diminished ability of basal cells to synthesize new basal lamina. Scallop formations occur since the detached basal lamina remains adherent to a contracting lamina propria. Basal lamina fusions take place. The changes of the basal lamina have been demonstrated also in other ultrastructural studies.

Vracko and Benditt11), studying reconstruction of skeletal muscle fibers and capillaries in rat and rabbit skeletal muscles after injury, reported that if the old basal lamina was separated from the plasma membrane of the new cell by some distance, then the new cell developed a new layer of basal lamina in apposition to its plasma membrane. Consequently, duplications of basal lamina occurred.

Although the exact mechanisms in producing changes of the basal lamina are not yet clear, it appeared from the results of the present study, as well as those of the previous investigations5,10,11), that the changes of the basal lamina as seen in Groups C and D were produced in the following manner. When the incisional trauma was applied to the gingiva, the basal lamina was first separated from the basal cells, then broke into fragments and moved downward into the connective tissue.

Fig. 10 Group D on Day 28. Normal basal lamina is seen just below the basal surface of the basal cell. But, in the underlying connective tissue, detachment, breaking, thickening, fragmentation, and multiplication of the basal lamina are also observed. (BC) : Basal Cell, (BL) : Basal Lamina, (AF) : Anchoring Fibril ×10,000.
While these processes were occurring the excess basal laminae began to exhibit various structural changes, many anchoring fibrils were attaching to one or both sides of these basal laminae. When bacterial protease was applied to the gingiva in addition to the incision, the structural changes of the basal lamina became more conspicuous.

In the present study, the changes as well as the amounts of the basal lamina in Groups C and D on Day 28 had decreased as compared with those in both groups on Day 4. These findings may indicate that reduction in the amount of irregular basal laminae was taking place in the gingival tissue with the passage of time. Vracko and Benditt(11) proposed that the removal of the irregular basal lamina was probably mediated by interstitial cells. But, in this study, no connective tissue cells were observed which were considered to be related with the removal or phagocytosis of the irregular basal laminae either on Day 4 or Day 28. So far, information concerning the fate of the changed basal lamina with the passage of time is scantly. Sodek and Ferrier(12) confirmed the existence of a remarkably high rate of collagen remodelling in the periodontal tissues in rats. This process might be related to the reduction of the basal lamina with the passage of time.

In the current study, in Group D on Day 4, the connective tissue cells, probably fibroblasts, were sometimes located near the basal cells or in direct contact with the basal cells of the gingiva. Since this situation was not observed in the gingiva of Group C on Day 4, it was probably brought about by the treatment of bacterial protease. However, the significance of this was not clear from the present investigation. Martinez(3) described that, 48 hours after incision of the gingiva, some fibroblasts were in close association with cytoplasmic projections of basal cells that extended into the fibrin mass. Tarin and Croft(13), investigating incised wound healing in mouse skin, reported that the dermal cells were in close contact with the epithelium on Day 5. These findings were also reported by Ross and Odland(14) who studied the connective tissue repair in human skin wounds. Tarin and Croft(13) postulated that the intimate relationship between dermal and epidermal cells in wound healing was possibly an important factor in controlling the epithelial invasion and in reestablishing epithelial differentiation.

In conclusion, the results of the present ultrastructural investigation suggested that the rat gingiva receiving wounds and bacterial protease showed conspicuous changes of the basal lamina at the epithelial-connective tissue junction and that these changes of the basal lamina were reduced in intensity with the passage of time.

References

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