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MIGRATION OF EPITHELIUM DURING PHENYTOIN-DEPENDENT GINGIVAL OVERGROWTH IN MICE

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Abstract

A small cavity was made in the mesiopalatal area of the maxillary first molar adjacent to the gingiva. Mice were maintained on 40 mg/kg phenytoin (or on diluent for control) by daily intraperitoneal injections. After 9 weeks, light microscopic observations revealed that in experimental mice, epithelial cells migrated towards the cavity and covered it. In controls, epithelial cell migration towards the cavity did not occur. For scanning electron microscopic (SEM) studies, specimens were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 2 hours, dehydrated, critical point dried and coated with gold. The surface of the outer gingival epithelium of experimental and of control mice showed a honeycomb arrangement of the microridges suggesting their keratinized nature. Epithelial cells lining the cavity showed well marked macroridges along their borders. Parallel microridges were observed on the upper surface of these cells suggesting that they were non-keratinized. It was concluded that the migrating epithelial cells, that covered the cavity during phenytoin-dependent gingival overgrowth, were of the non-keratinized type.

Key Words: Mice, phenytoin, phenytoin-dependent gingival overgrowth, gingiva, epithelium, enamel, cavity, scanning electron microscopy.

Introduction

Phenytoin (PHT) is still the anticonvulsant drug of choice for controlling seizures. One of the adverse reactions to PHT is phenytoin-dependent gingival overgrowth (PDGO), which was first reported by Kimball (1939). It is estimated that 2 million persons are given PHT every year to control their seizures, and approximately 50% of them develop PDGO (Stinnett et al., 1987). After 50 years of research, the etiological and pathological mechanisms by which phenytoin produces PDGO remain largely obscure. Various animal models have been investigated, e.g., ferrets (King and Gimson, 1947; Steinberg et al., 1972; Moore et al., 1979; Hall and Squier, 1982); cats (Ishakawa and Glickman, 1961; Nuki and Cooper, 1972; Hassell et al., 1982); monkeys (Staple et al., 1978) and guinea pigs (Carrel et al., 1983). All these animal models were expensive and difficult to handle. Recently, when plaque accumulation close to the gingiva was allowed, the rodent models demonstrated some success for phenytoin-dependent gingival overgrowth; Yamada et al. (1977), Do Nascimento et al. (1985) and Morisaki et al. (1990) used rats, and Sabet et al. (1990) used mice in their studies. In order to find a suitable and less expensive animal model, we investigated whether mice could be used successfully to study phenytoin-dependent gingival overgrowth (PDGO).

Materials and Methods

Animals

Thirty-six Balb C male mice, 5 weeks old, were purchased (Charles Rivers, Michigan). They were housed, 4 per cage, and given dry pellets (#300 by Agway Prolab) and water ad libitum.

Cavity Preparation

The animals were anesthetized by intraperitoneal injection of a mixture containing 10 µg of diazepam and 0.1 µl of Innovar Vet per gram body weight. Under the dissecting microscope, a small cavity was made with a 0.3 mm round burr in the mesiopalatal area of the
maxillary right first molar. The cavity was made in the middle of the molar, as close to the gingiva as possible, without touching the gingiva (Sabet et al., 1990). Plaques appeared in such cavities within a week.

Phenytoin

Phenytoin sodium (50 mg/ml) was purchased from Elkins-Sinn, Inc. It was diluted to 8 mg/ml with phosphate buffered saline, pH 11.0, to keep the phenytoin in solution.

Experimental design

After cavity preparation, mice were divided into two groups: an experimental group of 20 mice and a control group of 16 mice. Mice in the experimental group were kept on 40 mg/kg phenytoin by daily intraperitoneal injection starting from the day of cavity preparation and continuing throughout the 9 weeks of the study. Mice of the control group were similarly injected with the same volume (0.1 ml) of phosphate buffered saline, pH 11.0 (the phenytoin diluent). All mice were killed 9 weeks after cavity preparation.

Microscopy

Specimens from two mice of each group (experimental and control) were processed for routine light microscopy and specimens from eight mice of each group were processed for scanning electron microscopy (SEM). The remaining 16 animals (6 control and 10 experimental) were saved for further study. In each case, the maxillary area of the first molar was dissected out and fixed in cold 4% glutaraldehyde (made in 0.1 M phosphate buffer, pH 7.2) for 2 hours. They were then washed in phosphate buffer, dehydrated, dried using the critical point drying method, and coated with gold. The specimens were examined in a SEM operating at 15 kV. After SEM examination of the outer gingival surface near the cavity, maxillary first molars were removed from their sockets to examine the under-surface of the epithelial cell, which had migrated onto the cavity, to confirm whether they are keratinized or non-keratinized.

Results

Macroscopically, approximately 80% of the gingivae from mice treated with phenytoin (experimental) for 9 weeks showed gingival overgrowth covering the prepared cavity (Fig. 1) whereas none of the control animals showed gingival overgrowth to cover the cavity, although the cavity was filled with plaque (Fig. 2).

Light microscopy

The gingiva of the control group showed some signs of inflammation. Inflammatory cells were seen in both the epithelium and in the lamina propria, particularly in the area adjacent to the junctional epithelium. The blood vessels were dilated. The plaque formation in the prepared cavity was the source of the inflammation (Fig. 3). No sign of overgrowth was noticed in controls. The gingiva of the phenytoin-treated group showed overgrowth and the epithelial cells migrated onto the cavity on the tooth surface (Fig. 4). The gingival epithelium became hyperplastic, with increased numbers of rete pegs penetrating into the lamina propria. The lamina
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Figures 3 (above) and 4 (at right). Photomicrographs of demineralized sections showing the empty cavity (arrow) in the maxillary first molar of mouse from a control group (Fig. 3) after 9 weeks of cavity preparation; and filled with gingival epithelial tissue (arrow) after 9 weeks of phenytoin-treatment (Fig. 4). In Figure 3, note some increase in the thickness of the epithelial tissue but no migration of the epithelium into the cavity. In Figure 4, note a slight extension of epithelial processing (rete pegs) into the connective tissue region. Plaque (P); junctional epithelium (JE); sulcular epithelium (SE); outer gingiva (OG); tooth (T). Bars = 100 µm.

propria increased in thickness and contained some dilated blood vessels. Only a few inflammatory cells were observed.

Scanning electron microscopy (SEM)

The surface of the outer gingival epithelium from control specimens showed a regular honeycomb arrangement of uniform, smooth microridges covering micropits. The intercellular borders were composed of two macroridges (Fig. 5). The honeycomb arrangement of microridges was of similar pattern as seen at the cell surface of keratinized epithelium. The 9 weeks of phenytoin treatment did not alter the honeycomb pattern on the cell surface of the outer gingival epithelium. However, the microridges in PDGO were not of uniform thickness. They were fragile and covered pits of irregular shape (Fig. 6).

The free surface of the junctional epithelium from both control (Fig. 7) and experimental (Fig. 8) groups showed parallel microridges typical of non-keratinized epithelium. The parallel microridges in the experimental mice seemed to be closer to each other than in controls.

The superficial cell surface of the epithelium facing the cavity showed the same pattern of parallel microridges as in the non-keratinized epithelium. In the experimental group, when the maxillary first molar was removed from its socket, the epithelial cells lining the cavity were torn apart at the intercellular junction in most of the cases. Thus, it was possible to examine the under-surface of the epithelial cells lining the cavity. The under-surface of the epithelial cells showed distorted microvilli of different sizes (Fig. 9). Similarly, the surface of the cell originally attached below the separated epithelial cells showed a mirror image. The surface of these cells was covered with detached, distorted, and sometimes club-shaped microvilli (Fig. 10).

Discussion

The effect of PHT on the gingiva has been studied in humans and animals but the precise mechanism through which this drug acts on the gingival tissue is not fully understood (Hassell and Hefiti, 1991). In the past, scientists have used cats (Ishikawa and Glickman, 1961; Nuki and Cooper, 1972; Hassell et al., 1982); ferrets (King and Gimson, 1947; Hall and Squier, 1982); monkeys (Staple et al., 1978), and guinea pigs (Carrel et al.,
Figures 5 and 6. Scanning electron micrographs of the outer gingival surface from a control mouse (Fig. 5) and the outer gingival free surface from an experimental mouse (Fig. 6) showing honeycomb structures. In Figure 5, smooth microridges of uniform thickness surround the holes; double macroridges (arrow) indicate cell borders. In Figure 6, the microridges forming the honeycomb structure are not smooth and are not of uniform thickness. Bars = 2 µm.

Figure 7 and 8. Scanning electron micrographs of the junctional epithelium from a control mouse showing parallel microridges on the cell surface (Fig. 7); and near the cavity from an experimental mouse showing the free surface of the superficial cells (Fig. 8). In Figure 7, note the wider spacing of microridges (arrowheads) in comparison with experimental material (Fig. 8). In Figure 8, note the closer parallel microridges (arrowheads) on the cell surface than the control (Fig. 7) and prominent macroridges marking the cell border (arrow). Bars = 2 µm.
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The attempts to use rodents as an animals model for PDGO failed until Yamada et al. (1977) and Do Nascimento et al. (1985) successfully used rat models by inducing plaque accumulation near the gingiva. Since dental plaque accumulation on the tooth surface was found essential for the initiation of PDGO (Staple et al., 1978), plaque accumulation was induced in this study by making a cavity in the mesiopalatal area of the maxillary first molar adjacent to the gingiva and gingival overgrowth was induced by daily intraperitoneal injection of PHT in mice for 9 weeks.

The slight inflammation seen on light microscopic examination of the control and experimental gingival epithelium was due to plaque accumulation in the prepared cavity (Staple et al., 1978). The close relationship between local inflammation caused by dental plaque and severity of PDGO was also proposed by King and Gimson (1947) in their study of ferrets; this was later confirmed in various animals, including cats (Nuki and Cooper, 1972), and ferrets (Moore et al., 1979). In humans, PDGO has been associated with chronic gingival inflammation caused by dental plaque accumulation (Angelopoulos, 1975; Addy et al., 1983). The increase in the number of rete peg process observed in PDGO was also reported in human gingiva by Hassell (1981). Hyperplastic gingival epithelium was noticed in PDGO samples, indicating that PHT not only acts on gingival fibroblasts (Hassell et al., 1977, 1982; Hassell and Hefti, 1991) but also enhances the growth of gingival epithelium.

The outer surface of gingival keratinized epithelium showed a honeycomb structure in both the experimental and control specimens (Kaplan et al., 1977; Cleaton-Jones, 1975; Kullaa-Mikkonen, 1987). The microridges in the experimental specimens surrounding irregular holes or pits were not of uniform thickness; they looked delicate and fragile. This is in agreement with Dourov (1984), who reported that microridges surrounding micropits on the cell surface of PHT-treated human gingiva were of irregular elevation. The SEM examination of the junctional epithelium showed a parallel arrangement of microridges in the majority of the PDGO cases indicating its non-keratinized nature (Kaplan et al., 1977). The number of the parallel microridges was increased in

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Figure 9 (at left). Scanning electron micrograph of the separated under-surface of the epithelial cells lining the prepared cavity in an experimental mouse, showing distorted microvilli-like projections. Bar = 2 µm.

Figure 10 (at right). Scanning electron micrograph of the exposed superficial under-surface separated from to the cell shown in Figure 9. Note the complementary opposite surface, with detached distorted microvilli. Bar = 2 µm.
PHT-treated animals. The epithelial cell surface of the attached gingiva facing the tooth cavity showed parallel microridges similar to the type 2 pattern described by Moreu et al. (1993). They consisted of straight parallel rows of microridges as seen on the surface of the attached gingival epithelium. The under-surface of the detached epithelial cells from the gingival overgrowth covering the cavity showed detached and distorted microvilli and no depressions. The surface of the cell attached to the underlying cells revealed a mirror image arrangement of distorted microvilli. Cleaton-Jones (1975), McMillan (1979), and Satio and Itoch (1992) reported that the interconnecting microvilli of the under-surface of keratinized epithelial cells were surrounding the depressions. The opposing cell surfaces examined were found to be truly complementary to each other. Because these micrographs did not show a honeycomb appearance as in keratinized epithelium, therefore the under-surface seen in this study was composed of the non-keratinized epithelial cells. They might have migrated from the junctional epithelium.

We conclude that the PHT treatment and plaque accumulation in the cavity induced gingival overgrowth covering the cavity. SEM examination of the epithelial cell surface facing the cavity showed parallel microridges similar to those of non-keratinized epithelium. Previous studies of the PDGO focused on the lamina propria and fibroblasts. Our findings may have demonstrated scientific and clinical significance for studying the epithelium in PDGO. Also, the present investigation provides evidence that mice can be used as a suitable animal and less expensive model to investigate the cellular and molecular mechanisms of PDGO pathogenesis.

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References


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**Discussion with Reviewers**

**Reviewer I:** How do the investigators account for the increased frequency of parallel ridges noted in the experimental group?

**K. Arvidson:** Under Results, you mention "The parallel microridges in the experimental seemed to be closer to each other than in the control". Please present data?

**Authors:** Up-till now, most of the scientists showed the effect of phenytoin in connective tissue region but the present study indicates that phenytoin also effects epithelium. The significance of the present study is that there is a migration of the epithelium into the cavity in phenytoin treated animals and it attaches to the tooth surface in the presence of bacterial plaque.

**A. Campos:** Would you expect to find local variations in the SEM morphological surface pattern you saw in each area studied or did the cells in a given area (outer gingival epithelium, free surface of the sulcular epithelium, or the epithelium facing the cavity) to display the same pattern?

**Authors:** No, the surface of the outer gingiva and the sulcular epithelium showed honey-comb pattern, while the surface of the epithelium facing the cavity display parallel microridges.

**A. Campos:** What kind of quantitative studies do you think might be suitable for this material?

**Authors:** In future, we are planning to quantify the frequency of parallel microridges using point counting method; the data will be statistically analyzed using ANOVA and Student t-tests.

**K. Arvidson:** What kind of embedding material was used for the light microscopic study?

**Authors:** After decalcification of the tissues, paraffin embedding was used for the light microscopic study.

Reviewed by: K. Arvidson