Mini Review

Irsogladine maleate regulates gingival epithelial barrier function and intercellular communication in gingival epithelial cells

Tsuyoshi Fujita*, Hideki Shiba and Hidemi Kurihara

Department of Periodontal Medicine, Division of Frontier Medical Science, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan

Epithelial cells function as a mechanical barrier against invasion by pathogenic organisms and promote intercellular communication through cell-cell junction complexes. Therefore, the permeability and homeostasis of the gingival epithelial cell layer indicates a defensive capability against invasion by periodontal pathogens. Irsogladine maleate is a medication for gastric ulcer, and is also known to enhance gap junctional intercellular communication in cultured rabbit gastric epithelial and pancreatic cancer cells. We review the effects of Irsogladine maleate on barrier functions and gap junction intercellular communication of gingival epithelial cells under inflammatory conditions, to investigate the prevention of periodontal disease.

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*Correspondence should be addressed to:
Tsuyoshi Fujita, Department of Periodontal Medicine, Division of Frontier Medical Science, Hiroshima University Graduate School of Biomedical Sciences, Kasumi 1-2-3, Minami-ku, Hiroshima 734-8553, Japan. Phone: +81-82-257-5663, Fax: +81-82-257-5664, E-mail: tfuji@hiroshima-u.ac.jp

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Introduction

Periodontitis is an inflammatory condition caused by colonization of the gingival sulucus by periodontopathogenic bacteria. In periodontitis, gingival epithelial cells actively contribute to inflammatory processes as they represent the first line of defense against microbial attack. Epithelial cells function as a mechanical barrier against invasion by pathogenic organisms and promote intercellular communication through cell-cell junction complexes[1-4]. In addition, epithelial cells produce inflammatory cytokines and anti-microbial peptides. Therefore, the interaction between epithelial cells and periodontopathogenic bacteria and inflammatory cytokines has been suggested to play a significant role in the initiation of periodontitis.

The junctional epithelium is located at a strategically important interface at the base of the gingival sulcus. Although epithelial cells are generally interconnected by tight junctions, adherence junctions, desmosomes, and gap junctions, previous studies have shown that the junctional epithelium is interconnected only by a few desmosomes, and occasionally by gap junctions, and has wide intercellular spaces[5,6]. However, it was recently reported that claudin-1,

a tight junction structured protein, was expressed in the healthy junctional epithelium of Fischer 344 rats[7]. A previous report showed that claudin-1-deficient mice died within 1 day of birth and exhibited severe defects in the permeability of the epidermis[8]. Cells over-expressing claudin-1 showed increased trans-epithelial electrical resistance[9]. Therefore, claudin-1 may play an important role in the barrier function of the junctional epithelium, in spite of the absence of tight junctions. E-cadherin, a subclass of cadherin found in stratified squamous epithelium, plays a crucial role in maintaining the structural integrity and function of both adherens and desmosomal epithelial intercellular junctions[10]. In the junctional epithelium, E-cadherin is known to play an important role against bacterial invasion[11-13], although the reduction of E-cadherin was observed in inflamed gingival tissue[11,14]. In addition, *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans* decreased E-cadherin expression in cultured gingival epithelial cells[1,2]. In the gastric mucosal epithelium, the disruption of E-cadherin seems to cause epithelial permeability to increase[15]. Thus, the breakdown of interconnecting epithelial cell adhesions was suggested to lead to the disruption of the epithelial cell barrier function. Therefore, recovery of the barrier function may prevent bacterial invasion.

Gap junctions, whose structural proteins are connexins, are clusters of transmembranous hydrophilic channels that allow the direct exchange of molecules of up to 1,200 Da in weight, including ions, sugars, and small peptides, between adjacent cells[16,17]. Gap junctional intercellular communication plays a critical role in cellular coordination in tissue homeostasis.

Irsogladine maleate has been clinically used as an anti-gastric ulcer agent. Irsogladine maleate prevents gastric mucosal damage in several experimental animal models without inhibiting gastric secretion, and this prevention by Irsogladine maleate is related to improvement of the decrease in mucosal blood flow due to the disturbance of nitric oxide synthesis[18,19]. Irsogladine maleate enhances gap junctional intercellular communication in cultured rabbit gastric epithelial and pancreatic cancer cells through the augmentation of cyclic AMP[20,21]. Since Irsogladine maleate regulates the gastric epithelial function, it may be able to modulate the intercellular junctional complex on gingival epithelial cells. To review the effect and mechanism of Irsogladine maleate in gingival epithelial cells, we focused on the junctional complex and its function in the gingival epithelium.

The effect of Irsogladine maleate on gingival epithelium in an animal model

In an animal experiment, the application of *A. actinomycetemcomitans* to the gingival sulcus caused the dilatation of intercellular spaces, and the marked infiltration of polymorphonuclear leukocyte (PMNs) into the gingival epithelium. On the other hand, in Irsogladine maleate-injected rats, before *A. actinomycetemcomitans* application, the gingival epithelium showed the minimal migration of PMNs through intercellular spaces[22]. Immunohistochemical studies showed that the expression of E-cadherin was intensive in the junctional epithelium at cell-cell contacts from uninfected control rats and *A. actinomycetemcomitans-applied* rats under Irsogladine maleate pre-treatment. However, in *A. actinomycetemcomitans*-applied gingival epithelium, weaker staining for E-cadherin was observed in the junctional epithelium[22].

Irsogladine maleate regulates the permeability of human gingival epithelial cells (HGEC) by regulating E-cadherin and claudin-1

Although treatment with tumor necrosis factor (TNF)-α reduced transepithelial electrical resistance in HGEC, pretreatment with Irsogladine maleate prevented the TNF-α-induced reduction (Fig.1)[23]. In addition, using the fluorescein-dextran conjugate transport assay, we confirmed the effect of Irsogladine maleate on the permeability of HGEC stimulated by TNF-α. TNF-α also increased the concentration of fluorescein-dextran conjugate in the lower chamber, although Irsogladine maleate inhibited this increase[23]. Immunofluorescence staining showed that TNF-α affected the distribution of claudin-1 in HGEC, and the disrupted claudin-1 proteins were scattered in cytoplasmic compartments. In addition, Irsogladine maleate reversed the TNF-α-induced disruption in HGEC[23]. Furthermore, TNF-α suppressed the expression of E-cadherin at mRNA and protein levels, although Irsogladine maleate prevented the decrease. Immunofluorescence staining also indicated that Irsogladine maleate recovered the degradation of E-cadherin induced by TNF-α in HGEC[23]. The addition of *A. actinomycetemcomitans* to cultures also reduced the expression of E-cadherin at mRNA and protein levels. However, Irsogladine maleate, p38MAP kinase inhibitor, and

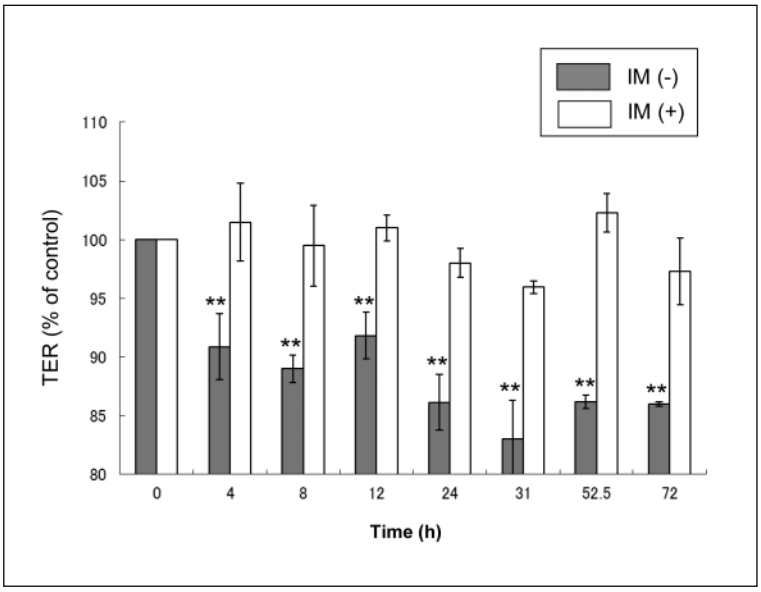

Fig.1 Effect of Irsogladine maleate on the permeability of the HGEC layer exposed to TNF-α

Transepithelial electrical resistance of the HGEC layer. Confluent HGEC on the polyethylene terephthalate membrane of a cell culture insert were pretreated with or without Irsogladine maleate at 1 μM for 1 h, and then exposed to TNF-α at 50 ng/ml for the indicated time. Transepithelial electrical resistance of HGEC was measured using Millicell-ERS.

**Differs significantly from control (0 h) (*t*-test, $p<0.01$).

extracellular signal-regulated kinase (ERK) inhibitor recovered the reduction in E-cadherin induced by *A. actinomycetemcomitans* at the mRNA and protein levels, suggesting that p38MAP kinase and ERK are involved in the reduction of E-cadherin[22]. In addition, the exposure of HGEC to *A. actinomycetemcomitans* induced the phosphorylations of p38 MAP kinase and ERK, and the addition of Irsogladine maleate inhibited both phosphorylations induced by *A. actinomycetemcomitans*[22]. Neither *A. actinomycetemcomitans* nor Irsogladine maleate affected the total ERK and total p38 MAP kinase.

Irsogladine maleate recovers the gap junctional intercellular communication and connexin43 in HGEC

The exposure of HGEC to *A. actinomycetemcomitans* decreased gap junction intercellular communication and connexin43 levels, whereas the simultaneous addition of Irsogladine maleate to the cultures abrogated these reductions[3]. Furthermore, Irsogladine maleate countered the interleukin (IL)-1β-induced suppression in gap junctional intercellular communication of HGEC[24]. Cyclic AMP and protein kinase A are known as intercellular signaling molecules induced by Irsogladine maleate, although its specific receptor remains unknown. Irsogladine maleate enhances gap junctional intercellular communication in cultured rabbit gastric cells and in pancreatic cancer cells though augmentation of cyclic AMP or protein kinase A[20,21]. In HGEC, Irsogladine maleate also countered the reduction in outer membrane protein29-induced gap junctional intercellular communication by up-regulating cyclic AMP levels[3].

Conclusion

Periodontitis is an inflammatory disease induced by bacterial biofilms that accumulate in the gingival sulcus. In addition to *A. actinomycetemcomitans*, endogenous gram-negative periodontal bacteria, such as *Porphyromonas gingivalis, Tannerella forsythia (forsythensis)*, and *Treponema denticola* initiate a series of aberrant inflammatory responses in periodontal tissue. These microbes possess numerous potent virulence factors neutralizing local host defenses and destroying periodontal tissues. Therefore, the further studies are necessary to elucidate the effect of Irsogladine maleate on the inflammation of gingival epithelium caused by the other periodontopathogenic bacteria.

Irsogladine maleate suppresses the bacteria- or cytokine-induced increase of IL-8 in HGEC, suggesting that Irsogladine maleate has an anti-inflammatory effect[3,4,22]. Although further studies are required, Irsogladine maleate, by regulating the physical barrier and homeostasis between epithelial cells in gingival epithelium, may be useful for the prevention of periodontitis (Fig.2).

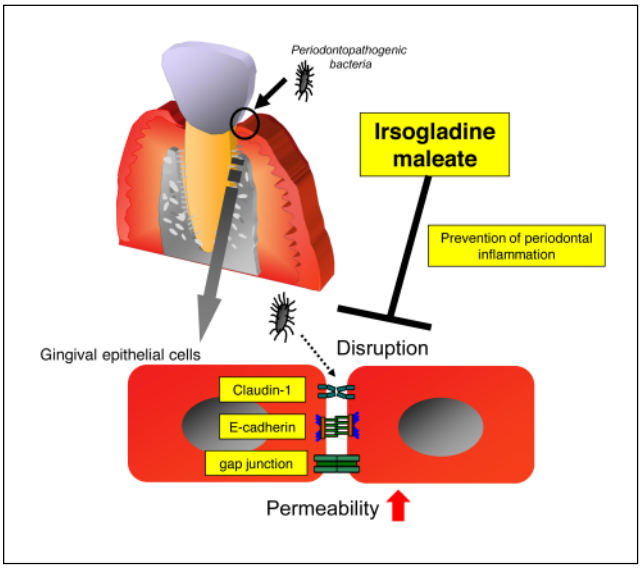

Fig.2
Irsogladine maleate, by regulating the gingival epithelial barrier function and intercellular communication in gingival epithelial cells, may be useful for the prevention of periodontal disease.

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