Review

Irsogladine maleate regulates barrier function and neutrophil accumulation in the gingival epithelium

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Abstract

Epithelial cells function as mechanical barriers against invasion by pathogenic organisms and promote intercellular communication through cell–cell junction complexes. Therefore, the permeability of the gingival epithelial cell layer indicates a defensive capability against invasion by periodontal pathogens. Accumulation of activated neutrophils is thought to be involved in the onset of inflammation. Here, we review the effects of irsogladine maleate, a medication for gastric ulcers, on E-cadherin and chemokine expression in gingival epithelial cells exposed to periodontopathogenic bacteria, in order to examine the clinical efficacy of irsogladine maleate in preventing periodontal inflammation.

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1. Introduction

Periodontitis is an inflammatory condition caused by colonization of the gingival sulcus by periodontopathogenic bacteria. In periodontitis, gingival epithelial cells actively contribute to inflammatory processes, since they represent the first line of defense against microbial attacks. Epithelial cells function as mechanical barriers against invasion by pathogenic organisms and promote intercellular communication through cell–cell junction complexes [1–3]. In addition, they produce inflammatory cytokines and anti-microbial peptides. Aggregatibacter actinomycetemcomitans is a major periodontopathogenic bacterium. Therefore, the interaction between epithelial cells and A. actinomycetemcomitans has been suggested to play an important role in the development of periodontitis.

E-cadherin, a key protein involved in the formation of desmosomes and adherens junctions, regulates the permeability of epithelial cells [4,5]. The gingival junctional epithelium is located at the base of the gingival sulcus, a strategically important interface. E-cadherin in the gingival junctional epithelium is known to play an important role against bacterial invasion [6], although a reduction in levels of E-cadherin was observed in inflamed gingival tissue [6,7]. In addition, Porphyromonas gingivalis or A. actinomycetemcomitans decreased E-cadherin expression in cultured gingival epithelial cells [2,3]. In the gastric mucosal epithelium, disruption of E-cadherin expression seems to increase epithelial permeability [8]. Thus, the breakdown of E-cadherin has been suggested to lead to the disruption of the epithelial cell barrier function. Recovery of barrier function may prevent bacterial invasion.

Chemotaxis of neutrophils to the site of infection is an important step in the immune response induced by chemoattractants, such as CXC chemokines CXCL-1 and CXCL-8 (interleukin [IL]-8). However, an accumulation of activated neutrophils in lesion areas is observed...
in all diseases and is thought to be involved in the onset of inflammation. The persistence of a local chronic host response may alter the protective roles of inflammatory cells and have deleterious effects on tissues [9–11]. In fact, the hyperactivity of neutrophils is associated with periodontal tissue destruction [12,13]. Further, it has also been reported that chronic inflammatory conditions often result from the aberrant production of chemokines such as IL-8 [14]. Aberrant IL-8 production can lead to chronic inflammatory conditions, as suggested for inflammatory diseases, such as rheumatoid arthritis [15,16]. Previous reports have shown that IL-8 is present in diseased human periodontal tissues [17–20], and the levels of IL-8 in both periodontal tissue and gingival crevicular fluid are correlated to disease severity [21]. In addition, the expression of IL-8 in diseased tissue, especially in the gingival epithelium, is correlated to the migration of polymorphonuclear leukocyte (PMNs) [22,23]. Therefore, blocking of excessive neutrophil activity and regulation of CXC-chemokine production may represent potential therapeutic strategies for inflammation.

Irsogladine maleate has been used clinically as an anti-gastric ulcer agent [24,25]. It has been shown to prevent gastric mucosal damage without inhibiting gastric secretion in several animal models [26–28]. Enhancement of gap junction intercellular communication through the activation of a muscarinic acetylcholine receptor and the increase in cyclic AMP levels in gastric epithelial cells by irsogladine maleate are involved in mucosal protection [29–32]. In addition, irsogladine maleate is also related to an improvement in mucosal blood flow that has been reduced because of a disturbance of nitric oxide synthesis [33]. Irsogladine maleate inhibits angiogenesis in vivo and in vitro [34–36]. Furthermore, it decreases superoxide production in human neutrophils [37] and IL-8 production in human gingival epithelial cells (HGECS) [1,38,39], suggesting that it has anti-inflammatory activity. Previous studies have shown that irsogladine maleate enhances gap junction intercellular communication through cyclic AMP in cultured gingival epithelial cells as well as in pancreatic cancer cells [1,40]. Irsogladine maleate may have multi-functions in gingival epithelial cells that afford protection from periodontal inflammation. We review the effect of irsogladine maleate on gingival epithelial cells, focusing on E-cadherin expression and the accumulation of neutrophils.

2. Effect of irsogladine maleate on E-cadherin expression in A. actinomycetemcomitans-stimulated HGECS

Immunohistochemical studies have shown marked expression of E-cadherin in the gingival junctional epithelium at cell–cell junctions in uninfected control rats and A. actinomycetemcomitans-infected rats pre-treated with irsogladine maleate. However, components of the A. actinomycetemcomitans-infected gingival junctional epithelium stained weakly for E-cadherin [41]. In vitro, the addition of A. actinomycetemcomitans to HGEC cultures reduced the expression of E-cadherin at the mRNA and protein levels. However, administration of irsogladine maleate, an inhibitor of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK), help recover the A. actinomycetemcomitans-induced reduction in E-cadherin expression at the mRNA and protein levels, suggesting that p38 MAPK and ERK are involved in the reduction of E-cadherin expression [41]. In addition, the exposure of A. actinomycetemcomitans to HGECS induced p38 MAPK and ERK phosphorylation.

**Fig. 1.** Irsogladine maleate regulates gingival epithelial barrier function and neutrophil migration to the gingival epithelium, suggesting its utility for preventing periodontal disease.
which was inhibited by irsogladine maleate [41]. Furthermore, pretreatment with irsogladine maleate prevented the tumor necrosis factor (TNF)-α-induced reduction of transepithelial electrical resistance in HGECS [42].

3. Effect of irsogladine maleate on chemokine production in A. actinomycetemcomitans-stimulated HGECS

In animal experiments, the inoculation of A. actinomycetemcomitans at the gingival sulcus caused dilatation of intercellular spaces and severe infiltration of the gingival epithelium by PMNs. Conversely, irsogladine maleate-injected rats showed minimal migration of PMNs through intercellular spaces [41]. An immunohistochemical study showed positive reactions for cytokine-induced neutrophil chemoattractant-2 (CINC-2) in a small number of gingival epithelial cells in control rats. A. actinomycetemcomitans-infected gingival epithelium stained strongly for CINC-2α, although irsogladine maleate pretreatment inhibited the A. Actinomycetemcomitans-induced increase in positive reactions for CINC-2α in gingival epithelial cells [41].

A DNA microarray analysis using cultures of HGECS suggested that the enhanced expression of CXCL-1, CXCL-2, CXCL-3, CXCL-6, and IL-8 was downregulated by irsogladine maleate in A. actinomycetemcomitans-stimulated HGECS. In fact, exposure to A. actinomycetemcomitans increased the levels of CXCL-1 and IL-8, and the addition of irsogladine maleate or an ERK inhibitor to the culture abolished this increase at the mRNA and protein levels. However, a p38 MAPK inhibitor had little effect on the A. actinomycetemcomitans exposure-induced increase in the mRNA expression of CXCL-1 and IL-8.

Compared to the unstimulated control, conditioned medium obtained from A. actinomycetemcomitans-stimulated HGECS enhanced human neutrophil chemotaxis in vitro. The co-incubation of either an anti-CXCR-1 or anti-CXCR-2 antibody with HGECS inhibited this chemotaxis. Furthermore, conditioned medium obtained from HGECS co-treated with A. actinomycetemcomitans and irsogladine maleate did not induce chemotactic activity, suggesting that irsogladine maleate reduces A. actinomycetemcomitans-induced neutrophil migration into the gingival epithelium by suppressing CXC-chemokine expression [41]. With regard to the inhibition of neutrophil migration, there is still some debate about host protection against tissue destruction. However, the persistence of a local chronic host response may alter the protective roles of inflammatory cells and have deleterious effects on tissues [9–11]. In fact, the use of anti-IL-8 neutralizing antibodies has led to clinically relevant reductions in disease activity among patients with palmoplantar pustulosis, a chronic inflammatory skin disease characterized by overexpression of IL-8 [43].

4. Conclusions

Although further studies are required, irsogladine maleate’s ability to regulate the function of the physical barrier between epithelial cells and neutrophil accumulation in the gingival epithelium suggests that it may be useful for preventing periodontal inflammation (Fig. 1).

Conflict of interest

No potential conflicts of interest are disclosed.

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