Journal of Oral Biosciences 54 (2012) 79–82

Contents lists available at SciVerse ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Review

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

Tsuyoshi Fujita*, Hideki Shiba, Hidemi Kurihara

Department of Periodontal Medicine, Division of Frontier Medical Science Hiroshima University Graduate School of Biomedical Sciences, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8553, Japan

ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form
9 February 2012
Accepted 14 February 2012
Available online 16 May 2012

Keywords:
Irsogladine maleate
Chemokine
E-cadherin
Gingival junctional epithelium

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.

Contents

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

* Corresponding author. Tel.: +81 82 257 5663; fax: +81 82 257 5664.
E-mail address: tfuji@hiroshima-u.ac.jp (T. Fujita).

1349-0079
http://dx.doi.org/10.1016/j.job.2012.02.001

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,

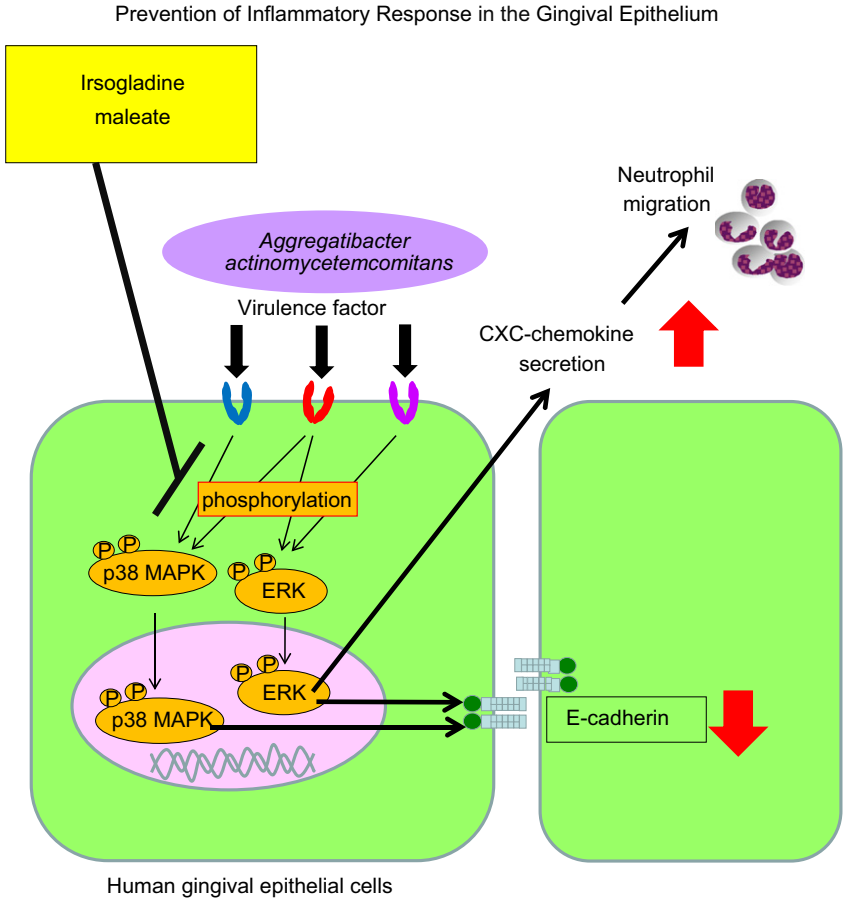

Fig. 1. Irsogladine maleate regulates gingival epithelial barrier function and neutrophil migration to the gingival epithelium, suggesting its utility for preventing periodontal disease.

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-2α) 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 HGEC 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 HGEC. 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 HGEC enhanced human neutrophil chemotaxis *in vitro*. The co-incubation of either an anti-CXCR-1 or anti-CXCR-2 antibody with HGEC inhibited this chemotaxis. Furthermore, conditioned medium obtained from HGEC 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.

Acknowledgments

We thank Nippon Shinyaku Co. Ltd., (Kyoto, Japan) for donating irsogladine maleate. We are also grateful to Dr. Yuushi Uchida, Kazuhisa Ouhara, Kouichi Hayashida, Mikihito Kajiya, Shinji Matsuda, Katsuhiro Takeda, Hiroyuki Kawaguchi, and Yoshimitsu Abiko for their support.

References

[1] Uchida Y, Shiba H, Komatsuzawa H, Hirono C, Ashikaga A, Fujita T, Kawaguchi H, Sugai M, Shiba Y, Kurihara H. Irsogladine maleate influences the response of gap junctional intercellular communication and IL-8 of human gingival epithelial cells following periodontopathogenic bacterial challenge. Biochem Biophys Res Commun 2005;333:502–7.
[2] Noguchi T, Shiba H, Komatsuzawa H, Mizuno N, Uchida Y, Ouhara K, Asakawa R, Kudo S, Kawaguchi H, Sugai M, Kurihara H. Syntheses of prostaglandin E2 and E-cadherin and gene expression of beta-defensin-2 by human gingival epithelial cells in response to *Actinobacillus actinomycetemcomitans*. Inflammation 2003;27:341–9.
[3] Katz J, Sambandam V, Wu JH, Michalek SM, Balkovetz DF. Characterization of *Porphyromonas gingivalis*-induced degradation of epithelial cell junctional complexes. Infect Immun 2000;68:1441–9.
[4] Niessen CM. Tight junctions/adherens junctions: basic structure and function. J Invest Dermatol 2007;127:2525–32.
[5] Wheelock MJ, Jensen PJ. Regulation of keratinocyte intercellular junction organization and epidermal morphogenesis by E-cadherin. J Cell Biol 1992;117:415–25.
[6] Ye P, Chapple CC, Kumar RK, Hunter N. Expression patterns of E-cadherin, involucrin, and connexin gap junction proteins in the lining epithelia of inflamed gingiva. J Pathol 2000;192:58–66.
[7] Hatakeyama S, Yaegashi T, Oikawa Y, Fujiwara H, Mikami T, Takeda Y, Satoh M. Expression pattern of adhesion molecules in junctional epithelium differs from that in other gingival epithelia. J Periodontal Res 2006;41:322–8.
[8] Wessler S, Backert S. Molecular mechanisms of epithelial-barrier disruption by *Helicobacter pylori*. Trends Microbiol 2008;16:397–405.
[9] Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J Periodontol 2003;74:391–401.
[10] Berglundh T, Donati M. Aspects of adaptive host response in periodontitis. J Clin Periodontol 2005;32(Suppl. 6):87–107.
[11] Kinane DF, Attström R, EWiPg B. Advances in the pathogenesis of periodontitis. Group B consensus report of the fifth European workshop in periodontology. J Clin Periodontol 2005;32(Suppl. 6):130–1.
[12] Del Fabbro M, Francetti L, Pizzoni L, Weinstein RL. Congenital neutrophil defects and periodontal diseases. Minerva Stomatol 2000;49:293–311.
[13] Waddington RJ, Moseley R, Embery G. Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. Oral Dis 2000;6:138–51.
[14] Harada A, Mukaida N, Matsushima K. Interleukin 8 as a novel target for intervention therapy in acute inflammatory diseases. Mol Med Today 1996;2:482–9.
[15] Seitz M, Dewald B, Gerber N, Baggiolini M. Enhanced production of neutrophil-activating peptide-1/interleukin-8 in rheumatoid arthritis. J Clin Invest 1991;87:463–9.
[16] Brennan FM, Zachariae CO, Chantry D, Larsen CG, Turner M, Maini RN, Matsushima K, Feldmann M. Detection of interleukin 8 biological activity in synovial fluids from patients with rheumatoid arthritis and production of interleukin 8 mRNA by isolated synovial cells. Eur J Immunol 1990;20:2141–4.
[17] Gamonal J, Acevedo A, Bascones A, Jorge O, Silva A. Characterization of cellular infiltrate, detection of chemokine receptor CCR5 and interleukin-8 and RANTES chemokines in adult periodontitis. J Periodontal Res 2001;36:194–203.
[18] Garlet GP, Martins W, Ferreira BR, Milanezi CM, Silva JS. Patterns of chemokines and chemokine receptors expression in different forms of human periodontal disease. J Periodontal Res 2003;38:210–7.
[19] Silva TA, Garlet GP, Fukada SY, Silva JS, Cunha FQ. Chemokines in oral inflammatory diseases: apical periodontitis and periodontal disease. J Dent Res 2007;86:306–19.
[20] Tonetti MS, Imboden MA, Gerber L, Lang NP, Laissue J, Mueller C. Localized expression of mRNA for phagocyte-specific chemotactic cytokines in human periodontal infections. Infect Immun 1994;62:4005–14.
[21] Tsai CC, Ho YP, Chen CC. Levels of interleukin-1 beta and interleukin-8 in gingival crevicular fluids in adult periodontitis. J Periodontol 1995;66:852–9.
[22] Miyauchi M, Kitagawa S, Hiraoka M, Saito A, Sato S, Kudo Y, Ogawa I, Takata T. Immunolocalization of CXC chemokine and recruitment of polymorphonuclear leukocytes in the rat molar periodontal tissue after topical application of lipopolysaccharide. Histochem Cell Biol 2004;121:291–7.
[23] Garlet GP, Avila-Campos MJ, Milanezi CM, Ferreira BR, Silva JS. *Actinobacillus actinomycetemcomitans*-induced periodontal disease in mice: patterns of cytokine, chemokine, and chemokine receptor expression and leukocyte migration. Microbes Infect 2005;7:738–47.
[24] Hiraishi H, Haruma K, Miwa H, Goto H. Clinical trial: irsogladine maleate, a mucosal protective drug, accelerates gastric ulcer healing after treatment for eradication of *Helicobacter pylori* infection-the results of a multicentre, double-blind, randomized clinical trial (IMPACT study). Aliment Pharmacol Ther 2010;31:824–33.
[25] Murakami K, Okimoto T, Kodama M, Tanahashi J, Mizukami K, Shuto M, Abe H, Arita T, Fujioka T. Comparison of the efficacy of irsogladine maleate

and famotidine for the healing of gastric ulcers after *Helicobacter pylori* eradication therapy: a randomized, controlled, prospective study. Scand J Gastroenterol 2011;46:287–92.
[26] Ueda F, Aratani S, Mimura K, Kimura K, Nomura A, Enomoto H. Effect of 2,4-diamino-6-(2,5-dichlorophenyl)-s-triazine maleate (MN-1695) on gastric mucosal damage induced by various necrotizing agents in rats. Arzneimittelforschung 1984;34:478–84.
[27] Ueda F, Aratani S, Mimura K, Kimura K, Nomura A, Enomoto H. Effect of 2,4-diamino-6-(2,5-dichlorophenyl)-s-triazine maleate (MN-1695) on gastric ulcers and gastric secretion in experimental animals. Arzneimittelforschung 1984;34:474–7.
[28] Sato M, Manabe N, Hata J, Ishii M, Kamada T, Kusunoki H, Shiotani A, Haruma K. Effect of irsogladine maleate on NSAID-induced reduction of gastric mucosal blood flow in anesthetized dogs. Digestion 2009;79:73–8.
[29] Ueda F, Ban K, Ishima T. Irsogladine activates gap-junctional intercellular communication through M1 muscarinic acetylcholine receptor. J Pharmacol Exp Ther 1995;274:815–9.
[30] Iwata F, Joh T, Ueda F, Yokoyama Y, Itoh M. Role of gap junctions in inhibiting ischemia-reperfusion injury of rat gastric mucosa. Am J Physiol 1998;275: G883–8.
[31] Ueda F, Watanabe M, Hirata Y, Kyoi T, Kimura K. Changes in cyclic AMP content of rat gastric mucosa induced by ulcerogenic stimuli-in relation to the antiulcer activity of irsogladine maleate. Jpn J Pharmacol 1991;55:493–9.
[32] Ueda F, Kyoi T, Mimura K, Kimura K, Yamamoto M. Intercellular communication in cultured rabbit gastric epithelial cells. Jpn J Pharmacol 1991;57:321–8.
[33] Kyoi T, Oka M, Noda K, Ukai Y. Irsogladine prevents monochloramine-induced gastric mucosal lesions by improving the decrease in mucosal blood flow due to the disturbance of nitric oxide synthesis in rats. J Pharmacol Sci 2003;93:314–20.
[34] Ren CJ, Ueda F, Roses DF, Harris MN, Mignatti P, Rifkin DB, Shapiro RL. Irsogladine maleate inhibits angiogenesis in wild-type and plasminogen activator-deficient mice. J Surg Res 1998;77:126–31.
[35] Sato Y, Morimoto A, Kiue A, Okamura K, Hamanaka R, Kohno K, Kuwano M, Sakata T. Irsogladine is a potent inhibitor of angiogenesis. FEBS Lett 1993;322:155–8.
[36] Ono M, Kawahara N, Goto D, Wakabayashi Y, Ushiro S, Yoshida S, Izumi H, Kuwano M, Sato Y. Inhibition of tumor growth and neovascularization by an anti-gastric ulcer agent, irsogladine. Cancer Res 1996;56:1512–6.
[37] Kyoi T, Noda K, Oka M, Ukai Y. Irsogladine an anti-ulcer drug, suppresses superoxide production by inhibiting phosphodiesterase type 4 in human neutrophils. Life Sci 2004;76:71–83.
[38] Fujita T, Ashikaga A, Shiba H, Uchida Y, Hirono C, Iwata T, Takeda K, Kishimoto A, Hirata R, Kawaguchi H, Shiba Y, Kurihara H. Regulation of IL-8 by irsogladine maleate is involved in abolishment of *Actinobacillus actinomycetemcomitans*-induced reduction of gap-junctional intercellular communication. Cytokine 2006;34:271–7.
[39] Kishimoto A, Fujita T, Shiba H, Komatsuzawa H, Takeda K, Kajiya M, Hayashida K, Kawaguchi H, Kurihara H. Irsogladine maleate abolishes the increase in interleukin-8 levels caused by outer membrane protein 29 from *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans* through the ERK pathway in human gingival epithelial cells. J Periodontal Res 2008;43:508–13.
[40] Kawasaki Y, Tsuchida A, Sasaki T, Yamasaki S, Kuwada Y, Murakami M, Chayama K. Irsogladine malate up-regulates gap junctional intercellular communication between pancreatic cancer cells via PKA pathway. Pancreas 2002;25:373–7.
[41] Fujita T, Kishimoto A, Shiba H, Hayashida K, Kajiya M, Uchida Y, Matsuda S, Takeda K, Ouhara K, Kawaguchi H, Abiko Y, Kurihara H. Irsogladine maleate regulates neutrophil migration and E-cadherin expression in gingival epithelium stimulated by *Aggregatibacter actinomycetemcomitans*. Biochem Pharmacol 2010;79:1496–505.
[42] Fujita T, Yumoto H, Shiba H, Ouhara K, Miyagawa T, Nagahara T, Matsuda S, Kawaguchi H, Matsuo T, Murakami S, Kurihara H. Irsogladine maleate regulates epithelial barrier function in tumor necrosis factor-α-stimulated human gingival epithelial cells. J Periodontal Res 2012;47:55–61.
[43] Skov L, Beurskens FJ, Zachariae CO, Reitamo S, Teeling J, Satijn D, Knudsen KM, Boot EP, Hudson D, Baadsqaard O, Parren PW, van de Winkel JG. IL-8 as antibody therapeutic target in inflammatory diseases: reduction of clinical activity in palmoplantar pustulosis. J Immunol 2008;181:669–79.