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ORIGINAL ARTICLE

Effects of polyhexamethylene guanidine phosphate on human gingival fibroblasts

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ABSTRACT

Objective: Polyhexamethylene guanidine phosphate (PHMG-P) was compared to chlorhexidine (CHX) in order to determine potential cytotoxic and immune-modulatory effects on human gingival fibroblasts.

Materials and methods: Cytotoxic effects of PHMG-P and CHX on human gingival fibroblasts were assessed using cell viability assay at various time points and concentrations. The effects of PHMG-P and CHX on the secretion of prostaglandin (PG) E_2, interleukin (IL)-6, IL-8 and matrix metalloproteinase (MMP)-1 by non-stimulated or IL-1β stimulated fibroblasts were evaluated by enzyme-linked immunosorbent assays.

Results: PHMG-P concentration 0.00009% led to the total loss of fibroblast viability within 24 h, whereas inhibition of fibroblast viability by CHX occurred at significantly higher concentrations of 0.0009% ($p < .001$). Short-term exposure to 0.005% PHMG-P led to loss of fibroblast viability after 5 min, whilst cells exposed to 0.005% CHX survived 30 min of treatment ($p < .001$). IL-1β stimulation induced an inflammatory response with a significant increase in the secretion of PGE_2, IL-6, IL-8 and MMP-1. Treatment of IL-1β stimulated fibroblasts in combination with PHMG-P or CHX at concentrations of 0.000045 or 0.0.00009% resulted in significantly decreased PGE_2, IL-6, IL-8 and MMP-1 levels. PHMG-P or CHX alone did not affect the baseline secretion of PGE_2, IL-6, IL-8 or MMP-1 by gingival fibroblasts.

Conclusions: Cytotoxic effects on gingival fibroblasts were triggered by both PHMG-P and CHX at concentrations below those used in clinical practice. The tested antiseptics did not cause inflammation and reduced IL-1β-induced secretion of inflammatory mediators and collagenase by gingival fibroblasts, which suggests anti-inflammatory properties.

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Introduction

Periodontitis is defined as inflammation of tooth supportive tissues caused by interaction between microbial biofilm and the host immune system. Untreated periodontitis may result not only in tooth loss but it may also affect general health, including increased risk of myocardial infarction and decreased quality of life [1,2]. Due to the infectious nature of the disease, antibacterial substances have become an essential component of periodontal treatment [3]. Chlorhexidine (CHX) is the most extensively used and tested antimicrobial in dentistry and it is still considered as the golden standard [4]. Antiseptics should meet a number of requirements, such as a broad microbicidal spectrum of activity with rapid onset and long-lasting effect, lack of toxicity towards host tissues/cells, and with minor interference into healing processes [5]. However, most antiseptic solutions display toxicity [6]. Muller and Kramer [7] introduced the biocompatibility index (BI) to ensure a more informative comparison between different antiseptics. BI is defined as the ratio of the mean concentration of the antiseptic agent allowing 50% survival (IC 50) of mouse fibroblasts L929 (ATCC CCL 1) after 30 min exposure at 37 °C against the mean concentration producing 3 $\log_{10}$ reduction in microbial colony-forming unit (*E. coli* and *S. aureus*) after 30 min exposure at 37 °C. Octenidine dihydrochloride and polyhexamethylene biguanide (PHMB) have proved to be the most suitable agents with BI over one [7].

Polyhexamethylene guanidine (PHMG) derivates, belonging to the polymeric guanidine family, have demonstrated high effectiveness in medicine and the food industry [8]. An expert meeting in 2008 recommended PHMB as a highly appropriate substance for use in critically colonized or infected acute and chronic wounds [9]. Structurally closely related to PHMB, PHMG phosphate (PHMG-P) has been proposed for applications in dentistry, although its toxicity with regard to gingival tissue requires assessment to determine optimal regimens of application in oral hygiene and dental disease treatment [10,11].

Currently, the role of PHMG-P in the containment of periodontitis may be attributed to its microbicidal effect on

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periopathogens, as well as on dental plaque-forming bacteria decreasing the bio-burden on gingiva and thereby reducing the inflammatory response [12]. A decline in inflammation may limit accumulation of early and late inflammatory cytokines and mediators, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, macrophage colony-stimulating factor (MCS-F), IL-17, prostaglandin (PG) E_2, and receptor activator of nuclear factor kappa-B ligand (RANKL) [13,14]. The accumulation of cytokines may increase the levels of osteoclast differentiation factor and osteoclastogenesis, giving rise to alveolar bone resorption and inhibition of osteogenic differentiation [15].

A study examining PHMG hydrochloride (PHMG-H) has shown a relatively low acute toxicity level (LD_{50} 600 mg/kg) in a rat model [16]. PHMG-P, which has minor structural differences compared to PHMG-H requires investigation regarding its toxicity towards human gingival fibroblasts and ability to increase or decrease inflammation. The aim of the current study was to investigate the cytotoxicity of PHMG-P compared to CHX, and their potential modulatory effects on secretion of inflammatory mediators and matrix metalloproteinase (MMP)-1 by human gingival fibroblasts.

Materials and methods

Antiseptics

PHMG-P 70% (w/v) gel (Institute of Eco-Technological Problems, Moscow, Russia) and 1% (w/v) CHX (Apotek Produktion & Laboratorier AB, Stockholm, Sweden) were utilized. Stock solutions for cell experiments were prepared ex tempore under sterile conditions and used immediately.

Fibroblast culture

Human gingival fibroblasts were isolated and cultured from gingival biopsies as previously described or purchased from CLS Cell Lines Service GmbH, Eppelheim, Germany [17]. The study was approved by the Regional Ethical Review Board in Stockholm. Briefly, minced gingival explants were transferred to 25 cm^3 Falcon tissue culture flasks containing 5 ml of Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen Life Technologies, Scotland, UK) supplemented with 4 mM L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin and 5% foetal calf serum (Invitrogen, Scotland, UK). The fibroblasts were cultured at 37 °C and 5% CO_2 in a humidified incubator and routinely passaged at 80% confluency using 0.025% trypsin in phosphate-buffered saline containing 0.02% EDTA (Invitrogen, Scotland, UK). Cells between passages 10 and 14 were used in the subsequent studies.

Cell viability assay

The effect of PHMG-P and CHX on cell viability was evaluated by determining metabolically active fibroblasts using a colorimetric procedure described by Mosmann [18]. Human gingival fibroblasts were seeded at 1×10^4 cells/well in 96-well tissue culture plates and cultivated for 24 h. The cells were rinsed twice with serum-free DMEM, followed by the addition of serum-free medium containing PHMG-P or CHX at concentrations 0.00005, 0.00009, 0.0005, 0.0009, 0.005, 0.009, 0.05, 0.1, 0.2, 0.5 and 1% w/v. The cells were incubated for another 24 h prior to the determination of cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer's instructions (Abnova, Taipei, Taiwan). The MTT assay is based on the ability of living cells to uptake and convert tetrazolium bromide to formazan via oxidoreductive mitochondrial function. Cells killed with saponin were used as a negative control. The cells were also treated with PHMG-P or CHX at 0.005, 0.05, 0.1, 0.2, 0.5 and 1% w/v for 1, 3, 15 and 30 min. Fibroblasts were rinsed twice with media supplemented with serum and analysed for cell viability. Optical density of the reaction product was measured by spectrophotometer (Labsystems Multiskan MS, Helsinki, Finland) at 540 nm. Cell viability was then scored according to the following formula: Cell viability (%) = (Optical density (OD) of the test group – OD of the positive control)/(OD of the negative control group – OD positive control) × 100. All cell culture experiments were carried out in two independent series and analysed in quadruplicate.

Stimulation of gingival fibroblasts with IL-1β

Effects of PHMG-P and CHX on the pro-inflammatory molecules PGE_2, IL-6, IL-8 and collagenase MMP-1 secreted by gingival fibroblasts were assessed in non-stimulated cells and cells stimulated with IL-1β.

Human gingival fibroblasts were seeded at a concentration 5×10^5 cells/well in 24-well plates. Following overnight incubation, cells were rinsed twice with serum-free media and treated with antiseptics CHX and PHMG-P at concentrations 0.000045% and 0.00009% (w/v) alone or in combination with IL-1β (300 pg/ml) (R&D Systems Inc., Minneapolis, MN) for 24 h. Serum-free DMEM or IL-1β served as negative and positive controls, respectively. Conditioned media supernatants were collected and stored at –80 °C for further analysis. The PGE_2, IL-6, IL-8 and MMP-1 levels in the supernatants were determined from conditioned media using commercial enzyme-linked immunosorbent assay (ELISA) kits for PGE_2 (Cayman Chemical, Ann Arbor, MI), IL-6, IL-8 and MMP-1 (R&D Systems Inc., Minneapolis, MN) according to the manufacturer's instructions. Absorbances were recorded at 450 nm on a microplate spectrophotometer (Labsystem Multiskan MS) with wavelength correction at 540 nm. Each antiseptic treatment was performed and analysed in triplicate.

Statistical analysis

Descriptive statistical data were computed and expressed as the mean ± standard deviation (SD). The differences between treatment groups were determined by one-way ANOVA combined with Tukey's multiple comparison test. p Value $\leq .05$ was regarded as statistically significant. Data processing was

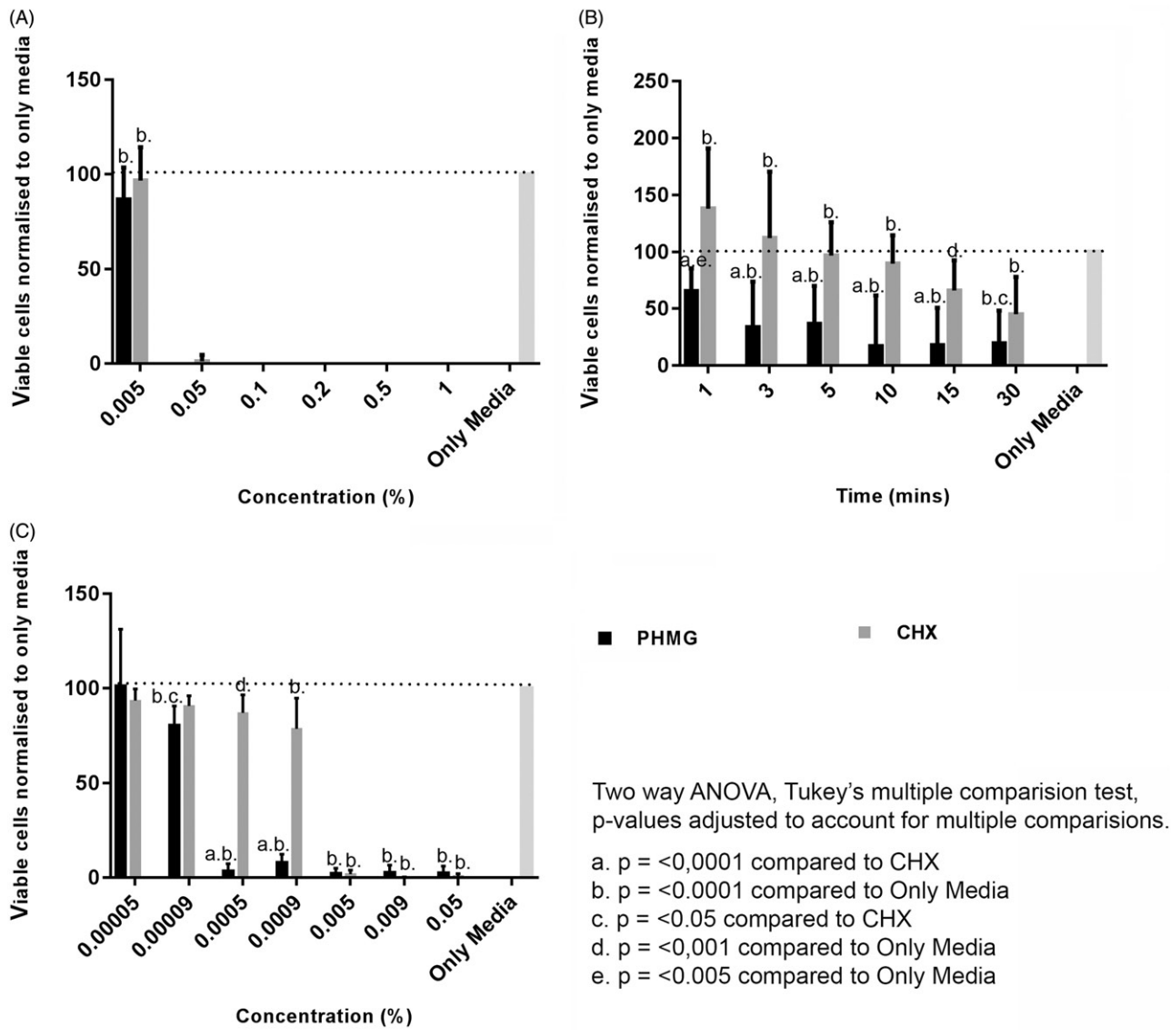

Figure 1. The effect of PHMG-P and CHX on cell viability of human gingival fibroblasts. (A) Viability of human gingival fibroblasts after 1 min of exposure to PHMG-P and CHX in the concentration range 0.005–1%. (B) Dynamics of gingival fibroblast viability after 30 min time course following treatment with either PHMG-P or CHX at 0.005%. (C) Viability of human gingival fibroblasts after 24 h of exposure to 0.00005–0.05% of PHMG-P and CHX. The differences between treatment groups were determined by one-way ANOVA combined with Tukey's multiple comparison test. *p* Value $\leq$.05 was regarded as statistically significant. All cell culture experiments were carried out in two independent series and analysed in quadruplicate.

performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA).

Results

Effect of PHMG-P and CHX on human gingival fibroblast viability

Evaluation of the effects of antiseptics on human gingival fibroblasts was performed by measuring the number of vital cells remaining after antiseptic administration over 24 h and in clinically relevant 1–30 min exposure time.

After 1 min of exposure, the maximum concentration of antiseptics retaining vital metabolic activity of human gingival fibroblasts was observed at 0.005% for both PHMG-P and CHX (Figure 1(A)). A prolonged exposure time (1–30 min) of cells to PHMG-P at 0.005% caused loss of viability of cells within 5 min, whilst cells subjected to 0.005% CHX retained viability even after 30 min exposure (Figure 1(B)). Long-term exposure (24 h) of cells to PHMG-P at concentrations higher than 0.00009% resulted in loss of fibroblast viability, whereas inhibition of fibroblasts viability by CHX occurred at concentrations higher than 0.0009% (Figure 1(C)).

Modulatory effect of PHMG-P and CHX on the secretion of inflammatory mediators and MMP-1 by human gingival fibroblasts

Human gingival fibroblasts were stimulated with IL-1β to induce an inflammatory response whereupon the effect of antiseptic administration of PHMG-P and CHX on the secretion of PGE_2, IL-6, IL-8 and MMP-1 was assessed. PHMG-P or CHX alone did not affect the levels of PGE_2, IL-6, IL-8 or

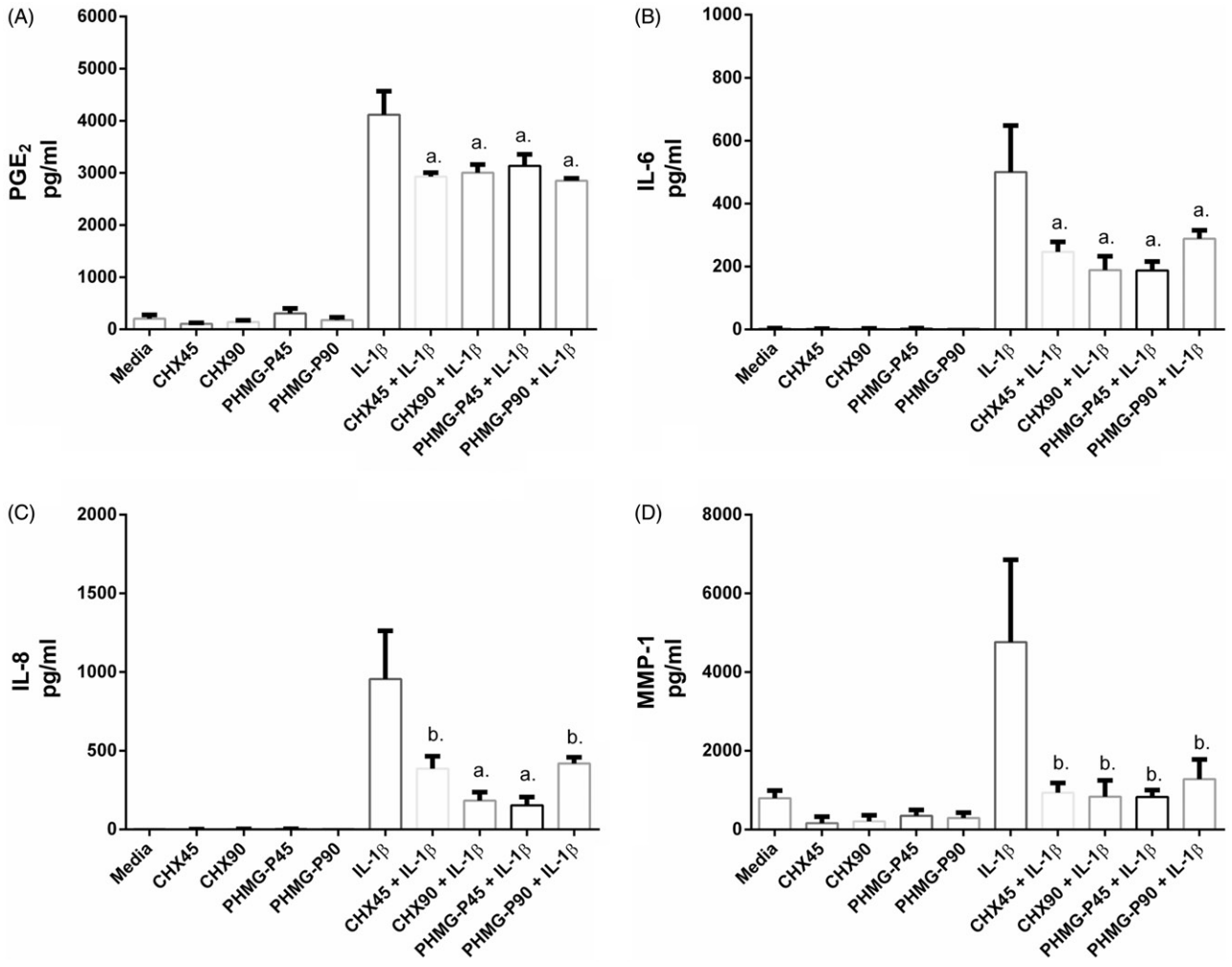

Figure 2. The effect of PHMG-P and CHX on the secretion of inflammatory mediators by human gingival fibroblasts. The levels of PGE_2 (A), IL-6 (B), IL-8 (C) and MMP-1 (D) secreted by non-stimulated or IL-1β-stimulated human gingival fibroblasts after treatment with PHMG-P or CHX (0.000045% and 0.00009%) were assessed in cell supernatants by ELISA. The differences between treatment groups were determined by one-way ANOVA combined with Tukey's multiple comparison test. p Value $\leq$.05 was regarded as statistically significant (a, $p \leq .001$ compared to IL1-β and b, $p \leq .005$ compared to IL-1β). Med: media only; CHX45: CHX 0.000045%; CHX90: CHX 0.00009%; PHMG-P45: PHMG-P 0.000045% and PHMG-P90: PHMG-P 0.00009%.

MMP-1 whereas, as expected, stimulation with IL-1β resulted in considerably increased levels of all four analysed mediators compared to untreated cells ($p < .0001$) (Figure 2(A–C)). Addition of PHMG-P or CHX along with IL-1β significantly reduced the levels of PGE_2 ($p < .001$), as well as IL-6, IL-8 and MMP-1 production by fibroblasts ($p < .05$) at all tested concentrations (Figure 2(A–D)).

Discussion

For development of new biocompatible antibacterial agents it is of utmost importance to assess both microbicidal activity and cytotoxic effect on human tissues. Decreasing inflammation during periodontitis can be achieved by diminishing the number of microorganisms, including periopathogenic, which implies application of antiseptics at concentrations that result in a significant reduction of microbial load on the periodontium [19]. Previously, we have verified the swift bactericidal activity of PHMG-P against periopathogenic bacteria at clinically-used concentrations and 20-fold dilutions [12]. In the current experiments, both PHMG-P and CHX influenced fibroblast viability at concentrations (0.005–0.00009%) lower than those applied in medical practice (0.05–1%) and those that displayed antimicrobial activity (0.05%) [12]. PHMG-P led to the loss of fibroblast viability over 24 h exposure at concentrations 10 times lower than CHX demonstrating that PHMG-P exhibited a stronger cytotoxic effect compared to the gold-standard CHX. These results are consistent with data of a previous study [20] which showed that PHMB was more toxic towards keratinocytes than CHX. The more potent cytotoxic effect of PHMG-P compared to CHX may be linked to its polymeric structure supporting enhanced adherence of the antiseptic to the tissue surface [21,22]. Nevertheless, loss of epithelial cells following antiseptic treatment should not be interpreted as an absolutely negative phenomenon, considering the key role of periopathogens intracellular survival in the pathogenesis of periodontitis [23]. To reduce cytotoxicity of PHMG-P, liposome supplements or oil-in-water emulsions containing egg yolk phosphatidylcholine could be added to the medicament [24].

Herein, we used *in vitro* gingival fibroblast culture, which does not truly recapitulate the human oral cavity environment, where different factors gradually reduce the

concentration of antiseptics, such as saliva and gingival crevicular fluid (GCF) [25]. Despite being toxic *in vitro*, PHMG-P has high water solubility, and therefore allows it to be easily diluted by saliva and GCF, and rapidly removed from periodontal pockets, thus preventing harsh effects of PHMG-P on epithelium [26]. PHMG-P not only interacts with the epithelium but also with the lamina propria fibroblasts. Scaling and root planing can damage the epithelial barrier of the periodontal pockets and an antiseptic solution could therefore directly affect the fibroblasts.

Molecules, less than 250 KDa or 40 Å, such as albumin, endotoxin, thymidine and histamine, may penetrate the epithelial lining via intercellular gaps reaching the connective tissue fibroblasts [27]. Indeed studies have shown [28] that CHX (0.2% w/v, Mw =505.446 g/mol) crossing of the basal membrane results in retention of CHX in the connective tissue at the non-toxic concentration of 1.5 pg, even after 24 h exposure. The molecular weight of PHMG-P varies from 408 to 956 g/mol, which would allow these molecules to pass through the mucosa and reach gingival fibroblasts, although it remains to be determined whether these non-toxic concentrations reduce inflammation by disrupting pro-inflammatory cytokines *in vivo* [29].

The present study showed immunomodulatory activity of PHMG-P and CHX applied at low concentrations. Röhner et al. [30] have previously reported the property of PHMB but not CHX to induce morphological cell damage after short exposure time, resulting in the secretion and accumulation of pro-inflammatory cytokines and chemokines, such as IL-1β, IL-6, IL-7 and TNF-α. However, in the current study, neither PHMG-P nor CHX provoked an inflammatory response from the gingival cells. Moreover, treatment of IL-1β stimulated human gingival fibroblasts with non-toxic concentrations of both PHMG-P and CHX led to statistically significant reduction of PGE_2, IL-6, IL-8 and MMP-1 levels. Previously, some antibiotics have been reported to downregulate the expression of pro-inflammatory cytokines IL-6, IL-8 and MMP-1 by fibroblasts [31].

The effect of antiseptics may be potentially justified by the ability to interact with proteins and impair their function, due to the chaotropic guanidine group in their structure, which is common to all biguanides, including PHMG-P [5,32,33]. CHX is known to substantially reduce the activity of commercial proteases (trypsin, chymotrypsin and proteinase K), cell-bound bacterial proteases (*P. gingivalis* and *T. denticola*) and MMP-2, 8 and 9 [34,35]. An *in vitro* study [36] has shown CHX to possess a strong short-term anti-inflammatory effect, reducing the levels of the pro-inflammatory cytokines, induced by *P. gingivalis*. Sakaki et al. [31] concluded that PGE_2 could also mediate IL-1β-stimulated expression of MMP-1. Inhibition of PGE_2 led to a decrease in MMP-1 production levels. Thus, it is plausible that both (PHMG-P and CHX) antiseptics could bind free IL-1β, PGE_2 or interfered in the pathway through which IL-1β upregulated inflammatory mediators, and consequently blocked the pro-inflammatory effect in human gingival fibroblasts resulting in the suppression of PGE_2, IL-6, IL-8 and MMP-1 production [37]. The antiseptics also may have bound IL-1β receptors preventing IL-1β mediated stimulation or they may have neutralized the released PGE_2, IL-6, IL-8 and MMP-1 [22,38]. However, further studies are required to elucidate the mechanisms of action but they are outside the scope of the current investigations.

Inactivation of early phase inflammatory cytokines by CHX or PHMG-P may arrest progression of the periodontal diseases. However, Türkoğlu et al. [39] failed to confirm a correlation between clinical improvement and diminished production of cytokines in GCF after CHX treatment. The interrelationship between polypeptide signalling mechanisms and tissue destruction during periodontitis pathogenesis confirms the importance of targeting inflammatory pathways, ranging from inflammatory cell recruitment and pro-inflammatory cytokines expression to RANKL-dependent osteoclastogenesis [40].

The current study shows that both CHX and PHMG-P can diminish concentration of cytokines during inflammation potentially providing additional benefit to the patient by exerting an immunomodulatory effect through arrest of pro-inflammatory molecule network, coupled to bactericidal effects, reduction in microbial and antigen load, and a moderate cytotoxic effect following exfoliation of epithelial cells burdened with periodontopathogenic bacteria [12,23].

Conclusions

Both PHMG-P and CHX displayed cytotoxic effects towards fibroblasts at concentrations lower than those used in clinical practice. PHMG-P exhibited higher toxicity within shorter time frame than CHX. PHMG-P and CHX displayed anti-inflammatory properties via reduction of IL-1β-induced secretion of PGE_2, IL-6, IL-8 and MMP-1 by gingival fibroblasts. PHMG-P as an antiseptic substance could be used for the control of periodontal diseases not only due to its antimicrobial activity, but also due to its property to reduce concentrations of pro-inflammatory mediators.

Disclosure statement

The study was performed in collaboration between Karolinska Institutet, Stockholm, Sweden and Belarusian State Medical University, Minsk, Belarus. This research was supported by the Swedish Institute Visby Programme (Grant number 00742/2010). No additional external funding was available for this investigation apart from that stated above and support of the authors' institutions. The authors declare that they have no conflicts of interest.

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References

[1] Rydén L, Buhlin K, Ekstrand E, et al. Periodontitis increases the risk of a first myocardial infarction A report from the PAROKRANK study. Circulation. 2016;133:576–583.

[2] Durham J, Fraser HM, McCracken GI, et al. Impact of periodontitis on oral health-related quality of life. J Dent. 2013;41:370–376.

[3] Hanes PJ, Purvis JP. Local anti-infective therapy: pharmacological agents. A systematic review. Ann Periodontol. 2003;8:79–98.
[4] Jones CG. Chlorhexidine: is it still the gold standard? Periodontol 2000. 1997;15:55–62.
[5] McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999;12:147–179.
[6] Mariotti AJ, Rumpf DA. Chlorhexidine-induced changes to human gingival fibroblast collagen and non-collagen protein production. J Periodontol. 1999;70:1443–1448.
[7] Muller G, Kramer A. Biocompatibility index of antiseptic agents by parallel assessment of antimicrobial activity and cellular cytotoxicity. J Antimicrob Chemother. 2008;61:1281–1287.
[8] Rosin M, Welk A, Bernhardt O, et al. Effect of a polyhexamethylene biguanide mouthrinse on bacterial counts and plaque. J Clin Periodontol. 2001;28:1121–1126.
[9] Dissemond J, Gerber V, Kramer A, et al. A practice-oriented recommendation for treatment of critically colonised and locally infected wounds using polyhexanide. J Tissue Viability. 2010;19:106–115.
[10] Ikeda T, Ledwith A, Bamford CH, et al. Interaction of a polymeric biguanide biocide with phospholipid membranes. Biochim Biophys Acta. 1984;769:57–66.
[11] Gilbert P, Moore LE. Cationic antiseptics: diversity of action under a common epithet. J Appl Microbiol. 2005;99:703–715.
[12] Vitt A, Sofrata A, Slizen V, et al. Antimicrobial activity of polyhexamethylene guanidine phosphate in comparison to chlorhexidine using the quantitative suspension method. Ann Clin Microbiol Antimicrob. 2015;14:36.
[13] Garlet GP. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. J Dent Res. 2010;89:1349–1363.
[14] Lee S-H, Kim T-S, Choi Y, et al. Osteoimmunology: cytokines and the skeletal system. BMB Rep. 2008;41:495.
[15] Behl Y, Siqueira M, Ortiz J, et al. Activation of the acquired immune response reduces coupled bone formation in response to a periodontal pathogen. J Immunol. 2008;181:8711–8718.
[16] Asiedu-Gyekye IJ, Mahmood SA, Awortwe C, et al. A preliminary safety evaluation of polyhexamethylene guanidine hydrochloride. Int J Toxicol. 2014;33:523–531.
[17] Yucel-Lindberg T, Olsson T, Kawakami T. Signal pathways involved in the regulation of prostaglandin E synthase-1 in human gingival fibroblasts. Cell Signal. 2006;18:2131–2142.
[18] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55–63.
[19] Stabholz A, Nicholas AA, Zimmerman GJ, et al. Clinical and antimicrobial effects of a single episode of subgingival irrigation with tetracycline HCl or chlorhexidine in deep periodontal pockets. J Clin Periodontol. 1998;25:794–800.
[20] Lee JE, Oum BS, Choi HY, et al. Cysticidal effect on acanthamoeba and toxicity on human keratocytes by polyhexamethylene biguanide and chlorhexidine. Cornea. 2007;26:736–741.
[21] Hwang HJ, Nam J, Yang SI, et al. MALDI-TOF analysis of polyhexamethylene guanidine (PHMG) oligomers used as a commercial antibacterial humidifier disinfectant. Bull Korean Chem Soc. 2013;34:1708–1714.
[22] Pantos A, Tsogas I, Paleos CM. Guanidinium group: a versatile moiety inducing transport and multicompartmentalization in complementary membranes. Biochim Biophys Acta (BBA)-Biomembr. 2008;1778:811–823.
[23] Rudney JD, Chen R, Sedgewick GJ. Intracellular *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in buccal epithelial cells collected from human subjects. Infect Immun. 2001;69:2700–2707.
[24] Muller G, Kramer A, Schmitt J, et al. Reduced cytotoxicity of polyhexamethylene biguanide hydrochloride (PHMB) by egg phosphatidylcholine while maintaining antimicrobial efficacy. Chem Biol Interact. 2011;190:171–178.
[25] Rathbone MJ, Tucker IG. Mechanisms, barriers and pathways of oral mucosal drug permeation. Adv Drug Deliv Rev. 1993;12:41–60.
[26] Dunn RL, Godowski KC, Harkrader RJ, et al. Mucosal delivery systems for treatment of periodontal disease. Google Patents. 1990.
[27] Tolo K. A study of permeability of gingival pocket epithelium to albumin in guinea pigs and Norwegian pigs. Arch Oral Biol. 1971;16:881IN11887IN13-886IN2888.
[28] Haugen E, Johansen JR. Penetration of the oral mucosa by radiolabelled chlorhexidine in guinea pigs. Acta Odontol Scand. 1975;33:365–372.
[29] Wei D, Ma Q, Guan Y, et al. Structural characterization and antibacterial activity of oligoguanidine (polyhexamethylene guanidine hydrochloride). Mater Sci Eng. 2009;29:1776–1780.
[30] Röhner E, Hoff P, Gaber T, et al. Cytokine expression in human osteoblasts after antiseptic treatment: a comparative study between polyhexanide and chlorhexidine. J Investig Surg. 2015;28:1–7.
[31] Sakaki H, Matsumiya T, Kusumi A, et al. Interleukin-1beta induces matrix metalloproteinase-1 expression in cultured human gingival fibroblasts: role of cyclooxygenase-2 and prostaglandin E2. Oral Dis. 2004;10:87–93.
[32] Hjeljord L, Rölla G, Bonesvoll P. Chlorhexidine–protein interactions. J Periodontal Res. 1973;8:11–16.
[33] Cserháti T, Szögyi M, Lelkes L. Charge-transfer chromatography used to study the interaction of chlorhexidine with proteins and amino acids. J Liq Chromatogr Relat Technol. 1995;18:2175–2192.
[34] Grenier D. Reduction of proteolytic degradation by chlorhexidine. J Dent Res. 1993;72:630–633.
[35] Gendron R, Grenier D, Sorsa T, et al. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clin Diagn Lab Immunol. 1999;6:437–439.
[36] Houri-Haddad Y, Halabi A, Soskolne WA. Inflammatory response to chlorhexidine, minocycline HCl and doxycycline HCl in an in vivo mouse model. J Clin Periodontol. 2008;35:783–788.
[37] Kida Y, Kobayashi M, Suzuki T, et al. Interleukin-1 stimulates cytokines, prostaglandin E2 and matrix metalloproteinase-1 production via activation of MAPK/AP-1 and NF-kappaB in human gingival fibroblasts. Cytokine. 2005;29:159–168.
[38] Cserháti T, Szögyi M, Lelkes L. Charge-transfer chromatography used to study the interaction of chlorhexidine with proteins and amino acids. J Liquid Chromatogr. 1995;18:2175–2192.
[39] Türkoğlu O, Becerik S, Emingil G, et al. The effect of adjunctive chlorhexidine mouthrinse on clinical parameters and gingival crevicular fluid cytokine levels in untreated plaque-associated gingivitis. Inflamm Res. 2009;58:277–283.
[40] Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. Trends Immunol. 2014;35:3–11.