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Effect of matrix metalloproteinase 8 inhibitor and chlorhexidine on the cytotoxicity, oxidative stress and cytokine level of MDPC-23

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ABSTRACT

Objective. This study investigated the effect of matrix metalloproteinase-8 inhibitor I (MMP8-I) and chlorhexidine (CHX) on the viability, oxidative stress and cytokine secretion of MDPC-23 under short-term (30 min) and long-term (3 days) culture.

Methods. MDPC-23 were treated with MMP8-I or CHX for 30 min, 1 day, 2 days and 3 days to detect the proliferation by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. In the following assays, MDPC-23 treated with 0.0003% CHX were referred to CHX group, treated with 8 μM MMP8-I were MMP8-I group. Cells without additional treatment were regarded as control group. The cell cycle, reactive oxygen species (ROS) level, and apoptosis were assessed by flow cytometry. The cytokine level was quantified by enzyme-linked immunosorbent assay (ELISA).

Results. In 30 min, CHX at concentrations higher than 0.0003% dilution inhibited cell proliferation when compared to the control group. MMP8-I (0.1–500 μM) showed no obvious cytotoxicity to MDPC-23, and MMP8-I (1000 μM) inhibited cell proliferation. In 3 days, CHX (0.0003%) significantly inhibited cell growth, while MMP8-I (8 μM) had no cytotoxicity. In the CHX group, the S phase population was decreased, and cellular ROS were increased in 3 days. In the MMP8-I group, the change of S phase population and cellular ROS was not significant compared with the control group. Cell apoptosis was not elevated in the MMP8-I group, while the apoptosis rate was increased in the CHX group both in 30 min and 3 days. In 30 min, CHX treatment significantly increased the secretion of interleukin (IL)-1β and IL-8, but slightly increased the secretion of IL-10, while MMP8-I caused no change in cytokines. In 3 days, CHX treatment significantly increased the secretion of IL-1β, IL-6, and IL-8, and inhibited the secretion of IL-10. MMP8-I treatment caused the increase of IL-6.

Significance. Compared with CHX, MMP8-I at low concentration did not result in cytotoxicity, oxidative stress, or the disorder of immune response.

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1. Introduction

The deterioration of resin–dentin bonds has become a focus of research in contemporary adhesive dentistry due to the proteolysis of collagen matrix of the hybrid layers [1]. Chlorhexidine (CHX), a nonspecific protease inhibitor of matrix metalloproteinase (MMP) and cysteine cathepsins, has been found to be beneficial to maintaining the integrity of the hybrid layer and preventing the loss of bond strength with time [2,3]. However, emerging evidence suggests that CHX exhibits both cytotoxicity and genotoxicity to oral cells, which greatly restricts its application in adhesive dentistry [4–6]. Recently, we reported that the synthetic MMP-8 inhibitor I (MMP8-I, 8 uM) protects the degradation of resin–dentin bonds over time [7]. Compared to CHX, MMP8-I is a selective inhibitor of MMP-8, which improved bond strength, preserved collagen integrity, and decreased nanoleakage after 1 year of in vitro storage [7]. In order to verify the security of MMP8-I application in adhesive dentistry, it is necessary to explore the effects of MMP8-I on odontoblast-like cells (MDPC-23).

During aerobic metabolism, cells regularly generate reactive oxygen species (ROS), including the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) [8,9]. Moderate amounts of ROS are considered to be signaling molecules in biological and physiological processes [10–12]. However, oxidative stress occurs when the generation of ROS disrupts the cellular redox homeostasis [11]. Oxidative stress plays an important role in delaying the cell cycle, increasing cell apoptosis, and modulating cytokines levels [13,14].

Cytokines, produced by a broad range of cells, are signaling proteins playing vital roles in biological phenomena, such as proliferation, inflammation, and immunomodulation [15,16]. Inflammation always accompanies the dysregulation of pro- and anti-inflammatory cytokines [16,17]. Therefore, cytokines reflect immune and cellular defensive responses when cells are destroyed by external factors. To our knowledge, the relationship between MMP8-I and cytokines remains unknown.

The aim of this study was to compare the effects of CHX and MMP8-I on the viability, oxidative stress, and cytokine secretion of MDPC-23. Because the MMP8-I was used as pretreatment, which is long-lasting in dental-resin interface, we explored the short-term (30 min) and long-term (3 days) effect of MMP8-I on MDPC-23.

2. Materials & methods

2.1. Culture of odontoblast-like cells

Immortalized odontoblast-like cells (MDPC-23) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco-BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco-BRL), 100 μg/mL streptomycin (HyClone, Logan, UT, USA), 100 U/mL penicillin (HyClone), and 5 mmol/L glutamine (Gibco-BRL) at 37 °C in 5% CO_2.

2.2. Cell proliferation assay

Cell proliferation of MDPC-23 was determined by MTT assay as described previously [18]. MDPC-23 were seeded at a density of 3000 cells per well and a volume of 100 μL of fresh medium in 96-well plates. CHX (Sigma, St. Louis, MO, USA) or MMP8-I (Merck KGaA, Darmstadt, Germany) was added in the culture medium at the various concentrations for different observation times (30 min, 1 day, 2 days and 3 days). Cells cultured in fresh medium without CHX or MMP8-I served as the negative control group. Briefly, 10 μL of MTT (5 mg/mL) solution was added to each well and incubated for 4 h at 37 °C. The supernatants from each well were discarded. Dimethyl sulfoxide (100 μL) was added and incubated for 10 min to dissolve the crystals. Cell number and viability were determined by measuring the absorbance at a wavelength of 490 nm with a microplate reader (Bio-Tek, Winooski, VT, USA).

2.3. Cell cycle analysis

For cell cycle analysis, each group was washed with cold PBS and fixed in 75% ethanol overnight at 4 °C. After being incubated with ribonuclease A (100 μg/mL in PBS), cells were stained with 50 μg/mL of propidium iodide (PI) at 4 °C for 30 min and analyzed using cytometry (Beckman Coulter, Krefeld, Germany). Data were analyzed using CytExpert software.

2.4. ROS staining

ROS staining was performed using an ROS staining kit (Beyotime, China) following the manufacturer's instructions [19]. MDPC-23 from each group were stimulated with serum-free medium containing 10 μM dichloro-dihydro-fluorescein diacetate (DCFH-DA) for 20 min at 37 °C, shaking slightly every 5 min. After removing the medium and washing the cells with serum-free culture medium, the cells were collected for examining the fluorescence intensity of each sample using flow cytometry (Beckman Coulter).

2.5. Apoptosis evaluation

The cytotoxic responses of MDPC-23 in the presence of CHX or MMP8-I were evaluated using cell apoptosis analyses. The MDPC-23 cultured in fresh medium were exposed to 0.0003% CHX or MMP8-I (8 μM) for 30 min and 3 days, while MDPC-23 cultured in fresh medium without CHX or MMP8-I were used as the negative control group. Cell apoptosis was analyzed using an PE Annexin V Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) following the manufacturer's protocol [20]. Briefly, 1×10^6 MDPC-23 were suspended in 1 × binding buffer, stained using PE-annexin V and 7-AAD staining solutions and analyzed by cytometry (Beckman Coulter).

2.6. Enzyme-linked immunosorbent assay (ELISA)

To quantify the secretory cytokines produced by MDPC-23, ELISA was performed according to the manufacturer's instructions. Briefly, after cells being cultured in each group for 30 min or 3 days, the supernatant was collected and analyzed with separate ELISA kits for mouse interleukin (IL)-1β, IL-6, IL-8, and IL-10 (NeoBioscience Technology, Guangdong, China). The

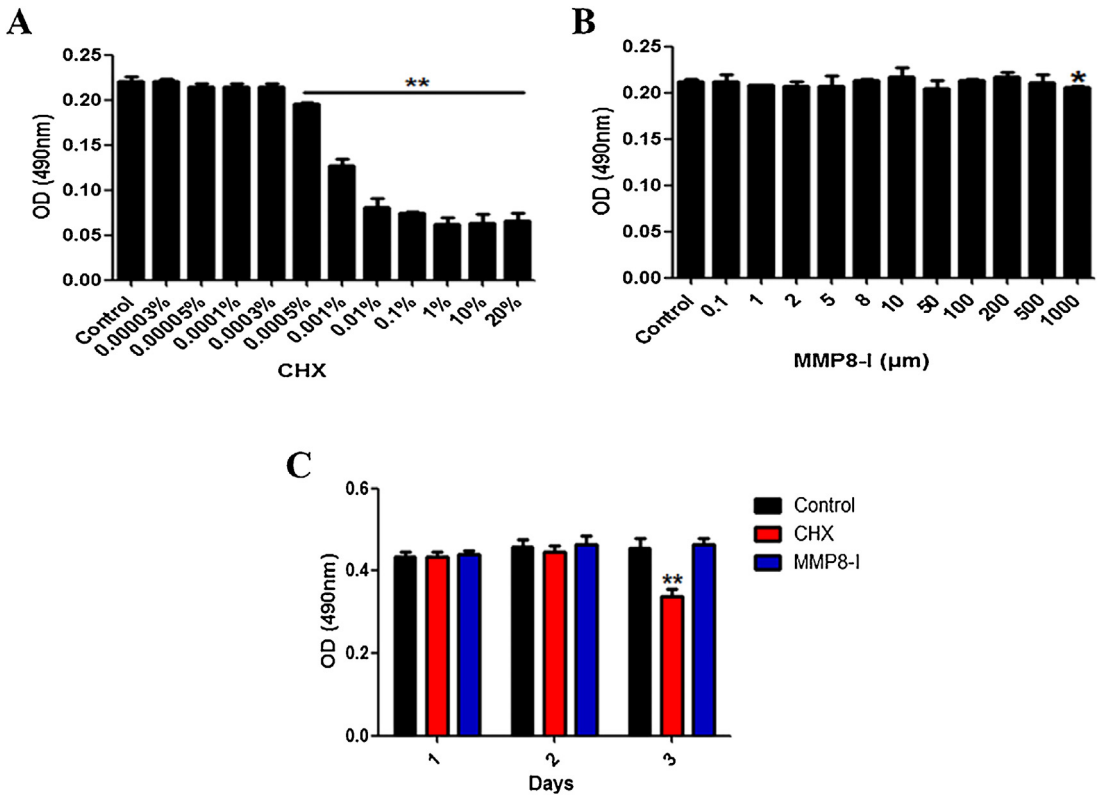

Fig. 1 – Assessment of effects on cell proliferation with CHX and MMP8-I.
MTT assay was performed after cells were treated with CHX (A) or MMP8-I (B) for short-term (30 min) and long-term (3 days) culture (C). All data are expressed as mean ± SD. *$p < 0.05$, **$p < 0.01$ compared with control group.

OD values were measured at 450 nm using a microplate reader (Bio-Tek).

2.7. *Statistical analysis*

All experiments were performed in triplicate. The SPSS 20.0 software package (SPSS, Chicago, IL, USA) was used for the statistical tests. Values are expressed as the mean ± SD. A one-way analysis of variance (ANOVA) with Tukey's test was performed to determine the statistical differences. In this study, $p < 0$ 05 was considered to indicate statistical significance.

3. Results

3.1. *Assessment of effects on cell viability with CHX and MMP8-I treatment*

For the MTT assay, cells were treated with CHX or MMP8-I at different concentrations for 30 min in order to evaluate cell growth. As the results showed, cells with CHX at concentration higher than 0.0003% had decreased cell proliferation when compared to the control group (Fig. 1A). No statistical differences were shown for MMP8-I treatment at concentrations ranging from 0.1 to 500 μM. However, 1000 μM MMP8-I may led to the decrease of cell proliferation (Fig. 1B). Then, we evaluated the cytotoxicity of long-term culture by treating cells with CHX (0.0003%) or MMP8-I (8 μM) and testing cell proliferation at 1, 2, and 3 days. CHX significantly inhibited cell growth at 3 days and MMP8-I treatment did not induce cytotoxicity (Fig. 1C). This indicated that CHX inhibited cell viability in dose- and time-dependent manners, while MMP8-I showed no cytotoxicity at concentration up to 500 μM.

3.2. *Assessment of cell cycle after CHX and MMP8-I treatment*

To further examine the cytotoxicity of CHX (0.0003%) and MMP8-I (8 μM) on MDPC-23, the cell cycle of different groups was detected. The results revealed that there was no significant difference in the S phase population among three groups for 30 min (Fig. 2A–C). In 3 days, the S phase population significantly decreased in the CHX group, while MMP8-I treatment did not decrease the S phase population of MDPC-23 (Fig. 2B–D).

3.3. *Measurement of ROS*

We further detected the effects of CHX (0.0003%) and MMP8-I (8 μM) treatment on the formation of intracellular ROS using a DCFH-DA probe. Neither CHX nor MMP8-I treatment elevated intracellular ROS in 30 min (Fig. 3A). However, in 3 days, CHX treatment caused the obvious increase of intracellular ROS, and MMP8-I treatment maintained the same level of ROS as the control group (Fig. 3B), which indicated that MMP8-I treatment did not induce cell oxidative stress.

3.4. *Assessment of cell apoptosis with CHX and MMP8-I treatment*

To test the effect of CHX (0.0003%) and MMP8-I (8 μM) treatment on cell apoptosis, PE-Annexin V/7AAD staining assay was performed. Our results showed that the percentage of apoptotic cells was significantly increased with CHX treatment for 30 min and 3 days, while MMP8-I treatment did not affect cell apoptosis in 30 min or 3 days (Fig. 4).

3.5. *Detection of chemokine secretion of cells treated with CHX or MMP8-I*

Finally, we tested the effect of CHX (0.0003%) and MMP8-I (8 μM) treatment on chemokine secretion of MDPC-23 by ELISA assay. Cells cultured with CHX treatment for 30 min significantly increased the secretion of IL-1β and IL-8, and did not influence the secretion of IL-6 and IL-10 (Fig. 5A). Treatment with MMP8-I for 30 min did not influence chemokine secretion of MDPC-23 compared to the control group (Fig. 5A). In 3 days, CHX increased the pro-inflammatory factors (IL-1β, IL-6 and IL-8) and decreased secretion of IL-10 (Fig. 5B). In the MMP8-I group, the secretion of IL-6 was increased (Fig. 5B).

4. Discussion

CHX has broad-spectrum antibacterial activity and can inhibit the catalytic activity of MMPs, so it's use is recommended as a cavity disinfectant to improve resin–dentin bond durability. However, CHX showed cytotoxic effects in MDPC-23, human osteoblastic, and fibroblastic cells [21–23]. Similarly, we found that CHX (0.005%–20%) is cytotoxic to MDPC-23 in short-term treatment (Fig. 1A). Therefore, cells are greatly damaged during the application of CHX in adhesive density.

MMP8-I, a selective inhibitor of MMP-8, based on the hydroxamate structure which specifically interferes with the action of the zinc catalytic domain in the MMP-8 molecule [24,25], protects against the degradation of resin–dentin bonds over time [7]. In our study, the cytotoxicity of CHX to MDPC-23 was dose- and time-dependent in short- and long-term treatment (Fig. 1A–C). MMP8-I at concentration up to 500 μM showed no cytotoxicity to MDPC-23 (Fig. 1B). The decrease of cell vitality is often connected with cell cycle arrest, and the S phase of the cell cycle is the phase in which DNA is replicated and the cells prepare for mitotic division [26,27]. CHX inhibits the viability of dermal fibroblasts by impeding

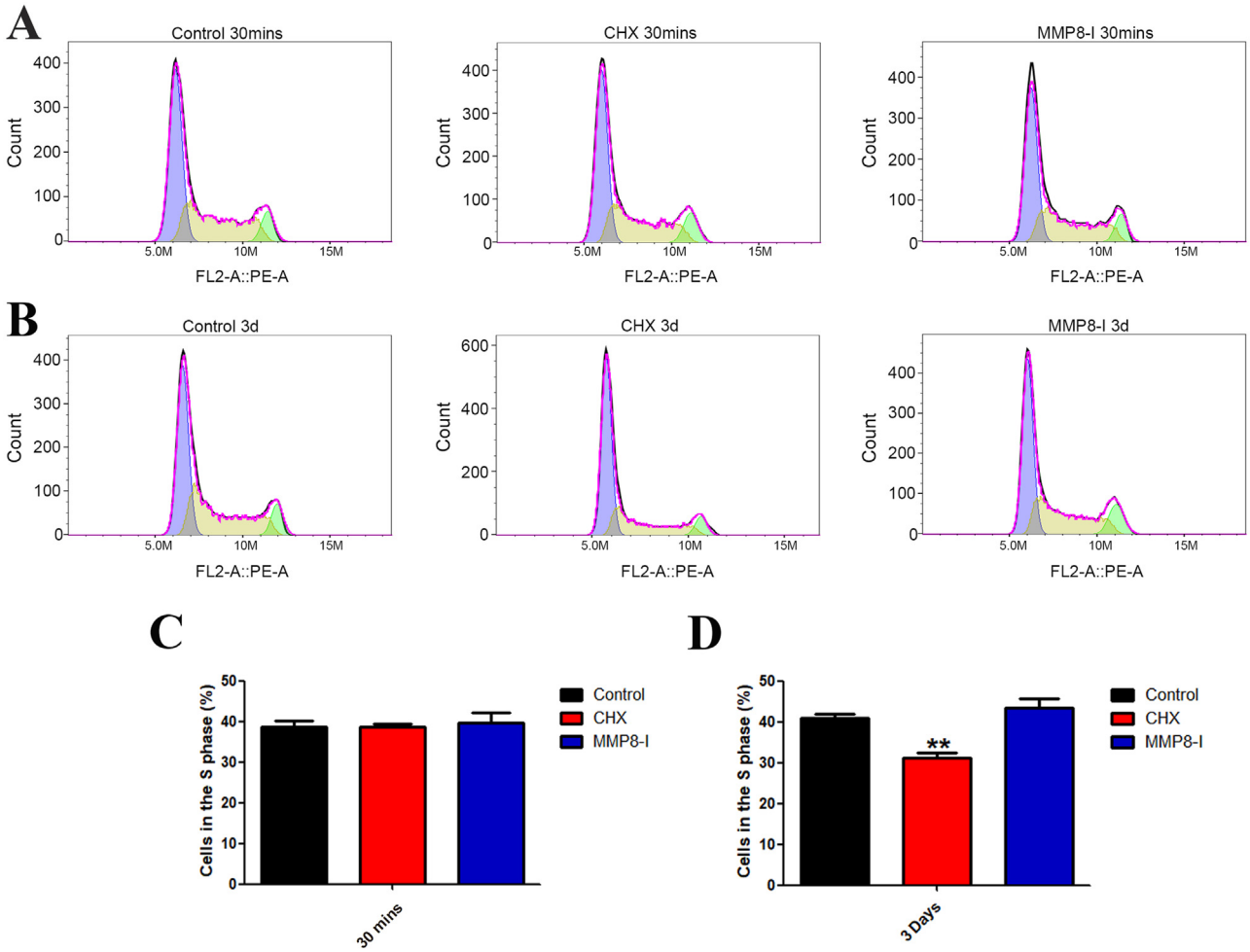

Fig. 2 – Cell cycle of MDPC-23.
Cell cycle of MDPC-23 in different groups in 30 min (A). Cell cycle of MDPC-23 in different groups in 3 days (B). Quantitative analysis of cell cycle (C and D). All data are expressed as mean ± SD. $^{**}p < 0.01$ compared with the control group.

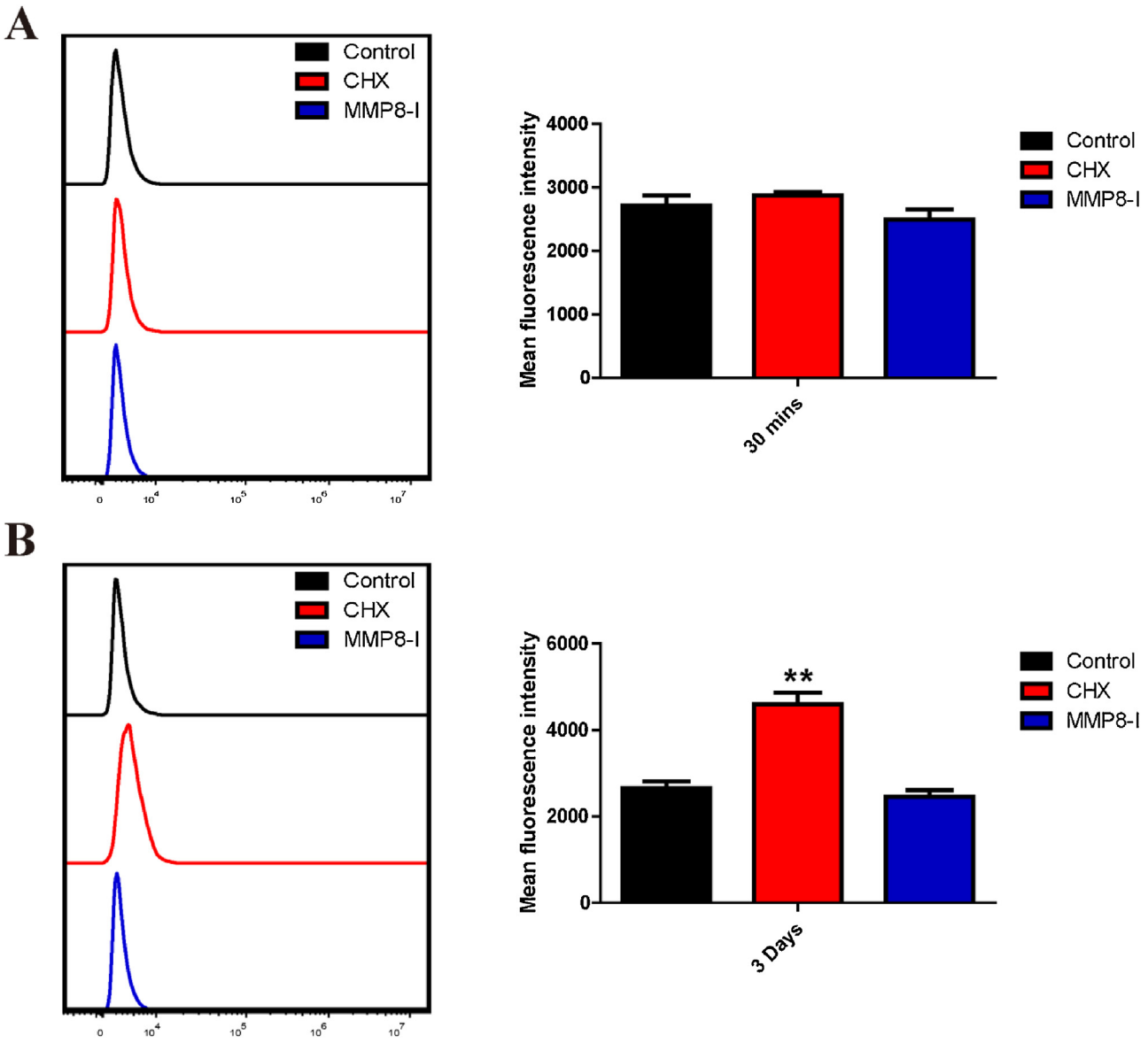

Fig. 3 – Measurement of intracellular ROS.
ROS production and quantitative analysis induced by CHX or MMP8-I treatment for 30 min (A) or 3 days (B). $^{**}p < 0.01$ compared with control group.

cells from advancing to the S phase [28]. In long-term treatment, the S phase population decreased in the CHX group, which inhibits cell proliferation through cell cycle arrest, while MMP8-I does not interrupt the cell cycle of MDPC-23 (Fig. 2). This demonstrated that MMP8-I is safer than CHX during the clinical application. The mechanism of CHX-induced cytotoxicity is associated with the increase of ROS and the induction of cell apoptosis [23,26].

ROS have been identified as chemical mediators in the processes of cell growth, cell apoptosis, and cytokine secretion. In osteoblasts, CHX induces cytotoxicity by promoting total ROS, which may cause further osteoblast impairment, such as cellular structure and DNA damage [29]. In MDPC-23, we found that CHX significantly increased the cellular ROS and apoptosis rate in 3 days (Figs. 3 and 4), while there was no difference between the MMP8-I group and the control group (Figs. 3 and 4), which further indicated that MMP-I had no obvious toxic effect on MDPC-23, possibly due to the concentration of MMP8-I and specificity to MMP-8 [30,31].

IL-1β is produced by many immune cells, including monocytes, macrophages, and dendritic cells, which is regarded as an early inflammatory biomarker [32,33]. In the CHX group, the levels of IL-1β were significantly increased both in 30 min and 3 days, while there were no significant changes between the MMP8-I group and the control group. This demonstrated that MMP8-I treatment did not cause early inflammation in MDPC-23. IL-6 is a pleiotropic cytokine produced by a variety of immune cells, which plays a role of both pro- and anti-inflammatory function in local immune response [34,35]. In CHX and MMP8-I groups, the levels of IL-6 were increased in 3 days, which showed that both CHX and MMP8-I stimulated the IL-6-related immune response in MDPC-23. Our

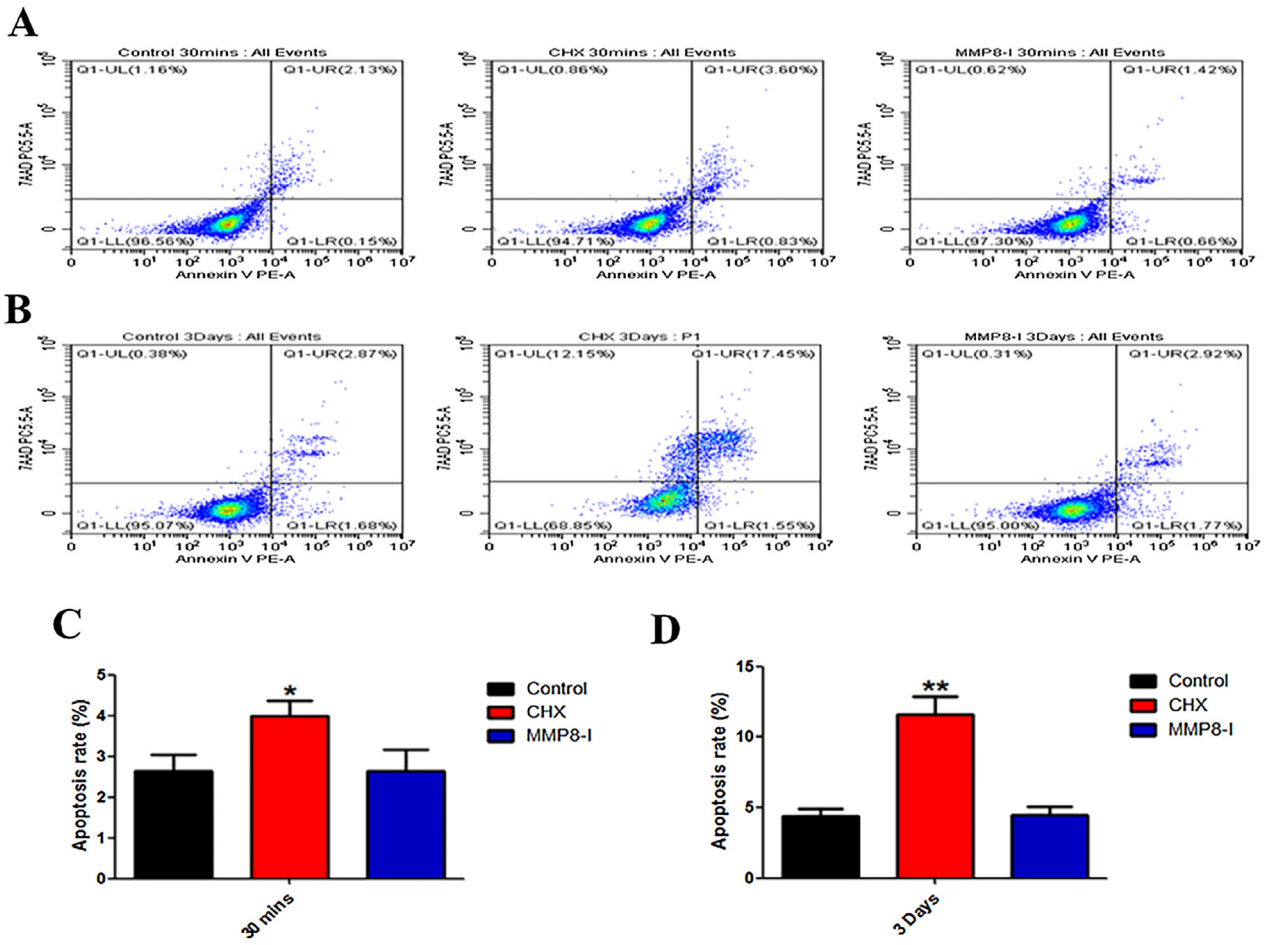

Fig. 4 – Assessment of cell apoptosis with CHX or MMP8-I treatment.
The cell apoptosis of MDPC-23 were treated with CHX or MMP8-I for 30 min (A) or 3 days (B). Quantitative analysis of apoptotic cells (C and D). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group.

results correspond to the previous study showing that both CHX and epigallocatechin-gallate increased the secretion of IL-6 [34]. IL-8 is another pro-inflammatory cytokine involving the chemotaxis of neutrophils [36,37]. Similar to the results of IL-1β, the production of IL-8 was enhanced in the CHX group, while MMP8-I caused no obvious change in IL-8 secretion, which further confirmed that MMP8-I caused more mild inflammatory response than CHX in MDPC-23. IL-10 is an anti-inflammatory cytokine, which plays a crucial role in immune defense [38]. CHX significantly inhibited the IL-10 secretion in 3 days, while the level of IL-10 in the MMP8-I group did not decrease, which showed that MMP8-I did not cause the disor-

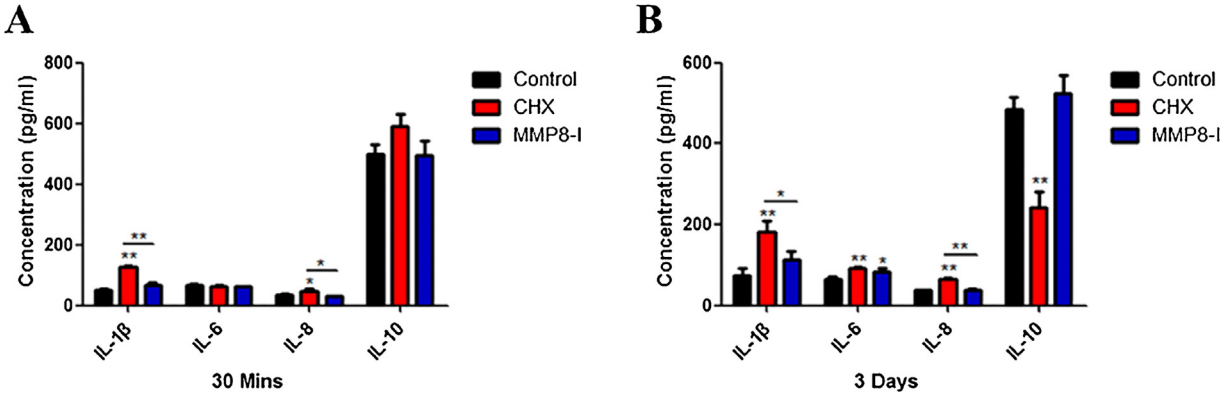

Fig. 5 – Chemokine secretion of cells treated with CHX or MMP8-I.
Cells were treated for 30 min (A). Cells were treated for 3 days (B). All data are expressed as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group.

der of the anti-inflammatory immune response. After treated for 30 min, MDPC-23 in CHX group secreted higher level of pro-inflammatory cytokine including IL-1β and IL-8. Cytokines did not present significant change in secretion levels in MMP8-I group. After treated for 3 days, cells in CHX group increased the level of pro-inflammatory cytokine (IL-1β and IL-8) and cytokine owning both pro- and anti-inflammatory function (IL-6). But the production of anti-inflammatory cytokine (IL-10) was decreased in the CHX group. In 3 days, IL-1β, IL-8 and IL-10 did not present significant change in secretion levels in MMP8-I group, the level of IL-6 was increased in MMP8-I group. The different patterns of cytokine secretion indicated CHX may be cytotoxic to MDPC-23 due to pro-inflammatory reaction, while MMP8-I is less toxic for MDPC-23.

Studies found that MMP-8 plays a pivotal role in arthritis, inflammatory and neurological disorders [39–41]. MMP-8 is upregulated in LPS-stimulated microglia, and the treatment of MMP8-I suppresses proinflammatory molecules, such as TNF-α [40]. In neuroinflammatory disorders, the treatment of MMP8-I decreases the secretion of ROS and proinflammatory molecules, particularly TNF-α, IL- 1β, and IL-6 [25].

Therefore, combined with the previous results [7], this study showed that MMP8-I is a promising adhesive in adhesive bonding systems due to its effectiveness of improving bond strength and low cytotoxicity in MDPC-23. In future research, because the caries-infected dentin is common in clinical practice, we will concentrate on the function of MMP8-I to dental pulp cells in order to explore the role of MMP8-I in dental bonding of caries-infected dentin. We will also focus on the improvement of drug-delivery strategy in order to extend the efficacy of MMP8-I, such as nanoparticle delivery carrier.

5. Conclusion

Compared with CHX, the application of MMP8-I caused lower cytotoxicity, oxidative stress, and the disorder of immune response, indicating that MMP8-I is less toxic than CHX for clinical application in adhesive dentistry.

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REFERENCES

[1] Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjaderhane L, et al. Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications. Dent Mater 2018;34:78.
[2] Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, Cenci MS. MMP inhibitors on dentin stability: a systematic review and meta-analysis. J Dent Res 2014;93:733.
[3] Stanislawczuk R, Reis A, Malaquias P, Pereira F, Farago PV, Meier MM, et al. Mechanical properties and modeling of drug release from chlorhexidine-containing etch-and-rinse adhesives. Dent Mater 2014;30:392.
[4] Tu YY, Yang CY, Chen RS, Chen MH. Effects of chlorhexidine on stem cells from exfoliated deciduous teeth. J Formos Med Assoc 2015;114:17.
[5] Pires CW, Botton G, Cadona FC, Machado AK, Azzolin VF, Da CI, et al. Induction of cytotoxicity, oxidative stress and genotoxicity by root filling pastes used in primary teeth. Int Endod J 2016;49:737.
[6] Priyadarshini BM, Selvan ST, Lu TB, Xie H, Neo J, Fawzy AS. Chlorhexidine nanocapsule drug delivery approach to the resin-dentin interface. J Dent Res 2016;95:1065.
[7] Ou Q, Hu Y, Yao S, Wang Y, Lin X. Effect of matrix metalloproteinase 8 inhibitor on resin-dentin bonds. Dent Mater 2018;34:756–63.
[8] Kanzaki H, Wada S, Narimiya T, Yamaguchi Y, Katsumata Y, Itohiya K, et al. Pathways that regulate ROS scavenging enzymes, and their role in defense against tissue destruction in periodontitis. Front Physiol 2017;8:351.
[9] Krifka S, Spagnuolo G, Schmalz G, Schweikl H. A review of adaptive mechanisms in cell responses towards oxidative stress caused by dental resin monomers. Biomaterials 2013;34:4555.
[10] Jha N, Ryu JJ, Choi EH, Kaushik NK. Generation and role of reactive oxygen and nitrogen species induced by plasma, lasers, chemical agents, and other systems in dentistry. Oxid Med Cell Longev 2017;2017:7542540.
[11] He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cell Physiol Biochem 2017;44:532.
[12] Chiu AV, Saigh MA, McCulloch CA, Glogauer M. The role of NrF2 in the regulation of periodontal health and disease. J Dent Res 2017;96:975.
[13] Su K, Wang CF, Zhang Y, Cai YJ, Zhang YY, Zhao Q. The inhibitory effects of carnosic acid on cervical cancer cells growth by promoting apoptosis via ROS-regulated signaling pathway. Biomed Pharmacother 2016;82:180.
[14] Wang H, Ao M, Wu J, Yu L. TNFalpha and Fas/FasL pathways are involved in 9-methoxycamptothecin-induced apoptosis in cancer cells with oxidative stress and G2/M cell cycle arrest. Food Chem Toxicol 2013;55:396.
[15] Zahari W, Hashim SN, Yusof MF, Osman ZF, Kannan TP, Mokhtar KI, et al. Immunomodulatory effect of cytokines in the differentiation of mesenchymal stem cells: a review. Curr Stem Cell Res Ther 2017;12:197.
[16] Yazid FB, Gnanasegaran N, Kunasekaran W, Govindasamy V, Musa S. Comparison of immunodulatory properties of dental pulp stem cells derived from healthy and inflamed teeth. Clin Oral Investig 2014;18:2103.
[17] Morand DN, Davideau JL, Clauss F, Jessel N, Tenenbaum H, Huck O. Cytokines during periodontal wound healing: potential application for new therapeutic approach. Oral Dis 2017;23:300.
[18] Da FRGL, Pontes EC, Basso FG, Hebling J, de Souza CC, Soares DG. Transdentinal cytotoxicity of resin-based luting cements to pulp cells. Clin Oral Investig 2016;20:1559.
[19] Schweikl H, Gallorini M, Forstner M, Petzel C, Bolay C, Hiller KA, et al. Flavin-containing enzymes as a source of reactive oxygen species in HEMA-induced apoptosis. Dent Mater 2017;33:e255.
[20] Schweikl H, Widbiller M, Krifka S, Klement J, Petzel C, Bolay C, et al. Interaction between LPS and a dental resin monomer on cell viability in mouse macrophages. Dent Mater 2016;32:1492.
[21] de Souza LB, de Aquino SG, de Souza PP, Hebling J, Costa CA. Cytotoxic effects of different concentrations of chlorhexidine. Am J Dent 2007;20:400.
[22] Lee TH, Hu CC, Lee SS, Chou MY, Chang YC. Cytotoxicity of chlorhexidine on human osteoblastic cells is related to intracellular glutathione levels. Int Endod J 2010;43:430.

[23] Giannelli M, Chellini F, Margheri M, Tonelli P, Tani A. Effect of chlorhexidine digluconate on different cell types: a molecular and ultrastructural investigation. Toxicol In Vitro 2008;22:308.
[24] Matter H, Schwab W, Barbier D, Billen G, Haase B, Neises B, et al. Quantitative structure-activity relationship of human neutrophil collagenase (MMP-8) inhibitors using comparative molecular field analysis and X-ray structure analysis. J Med Chem 1999;42:1908.
[25] Lee EJ, Choi MJ, Lee G, Gaire BP, Choi JW, Kim HS. Regulation of neuroinflammation by matrix metalloproteinase-8 inhibitor derivatives in activated microglia and astrocytes. Oncotarget 2017;8:78677.
[26] Li YC, Kuan YH, Lee SS, Huang FM, Chang YC. Cytotoxicity and genotoxicity of chlorhexidine on macrophages in vitro. Environ Toxicol 2014;29:452.
[27] Bakopoulou A, Leyhausen G, Volk J, Papachristou E, Koidis P, Geurtsen W. Wnt/beta-catenin signaling regulates Dental Pulp Stem Cells' responses to pulp injury by resinous monomers. Dent Mater 2015;31:542.
[28] Hidalgo E, Dominguez C. Mechanisms underlying chlorhexidine-induced cytotoxicity. Toxicol In Vitro 2001;15:271.
[29] Proksch S, Strobel SL, Vach K, Abouassi T, Tomakidi P, Ratka-Kruger P, et al. Melatonin as a candidate therapeutic drug for protecting bone cells from chlorhexidine-induced damage. J Periodontol 2014;85:e379.
[30] Feng Q, Shabrani N, Thon JN, Huo H, Thiel A, Machlus KR, et al. Scalable generation of universal platelets from human induced pluripotent stem cells. Stem Cell Rep 2014;3:817.
[31] Zhou Y, Liu X, Xu L, Tseng H, Cao Y, Jiang J, et al. Matrix metalloproteinase-8 is overexpressed in Waldenstrom's macroglobulinemia cells, and specific inhibition of this metalloproteinase blocks release of soluble CD27. Clin Lymphoma Myeloma Leuk 2011;11:172.
[32] Sawada K, Fujioka-Kobayashi M, Kobayashi E, Schaller B, Miron RJ. Effects of antiseptic solutions commonly used in dentistry on bone viability, bone morphology, and release of growth factors. J Oral Maxillofac Surg 2016;74:247.
[33] Jun SK, Kim HW, Lee HH, Lee JH. Zirconia-incorporated zinc oxide eugenol has improved mechanical properties and cytocompatibility with human dental pulp stem cells. Dent Mater 2018;34:132.
[34] Stavroullakis AT, Carrilho MR, Levesque CM, Prakki A. Profiling cytokine levels in chlorhexidine and EGCG-treated odontoblast-like cells. Dent Mater 2018;34:e107–14.
[35] Jerg A, Schulz SD, Tomakidi P, Hellwig E, Polydorou O. Modulation of gingival cell response towards dental composites. Dent Mater 2018;34:412.
[36] Nakayama M, Ohara N. Molecular mechanisms of *Porphyromonas gingivalis*-host cell interaction on periodontal diseases. Jpn Dent Sci Rev 2017;53:134.
[37] Czepielewski RS, Jaeger N, Marques PE, Antunes MM, Rigo MM, Alvarenga DM, et al. GRPR antagonist protects from drug-induced liver injury by impairing neutrophil chemotaxis and motility. Eur J Immunol 2017;47:646.
[38] Hotchkiss KM, Ayad NB, Hyzy SL, Boyan BD, Olivares-Navarrete R. Dental implant surface chemistry and energy alter macrophage activation in vitro. Clin Oral Implants Res 2017;28:414.
[39] Dejonckheere E, Vandenbroucke RE, Libert C. Matrix metalloproteinase8 has a central role in inflammatory disorders and cancer progression. Cytokine Growth Factor Rev 2011;22:73.
[40] Lee EJ, Han JE, Woo MS, Shin JA, Park EM, Kang JL, et al. Matrix metalloproteinase-8 plays a pivotal role in neuroinflammation by modulating TNF-alpha activation. J Immunol 2014;193:2384.
[41] Han JE, Lee EJ, Moon E, Ryu JH, Choi JW, Kim HS. Matrix metalloproteinase-8 is a novel pathogenetic factor in focal cerebral ischemia. Mol Neurobiol 2016;53:231.