Original Article

Antibacterial peptides inhibit MC3T3-E1 cells apoptosis induced by TNF-α through p38 MAPK pathway

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Background: Antimicrobial peptides (AMP), as a small molecular polypeptide with a broad antibacterial spectrum and high efficiency, have attracted more and more attention. Few pieces of research on the effect of the antimicrobial peptide on osteoblast under inflammatory conditions have so far been reported. The main aim of this work was to investigate the antiapoptosis effect of the antimicrobial peptide on MC3T3-E1 cells induced by TNF-α and its related mechanism.

Methods: Rat MC3T3-E1 cells were co-cultured with different concentrations of antibacterial peptide DP7 and TNF-α.MTS assay, cell scratch test, alkaline phosphatase activity, and alizarin red staining assay were used to determine osteoblast viability in this experiment. Annexin V-FITC/PI double staining cells and flow cytometry were used to analyze apoptosis and Western blot assay detection to show mitogen-activated protein kinase (MAPK) protein expression in rat MC3T3-E1 cells. Then, Realtime polymerase chain reaction (PCR) was used to examine the caspase-3 gene expression. Also, ELISA detection was used to clarify the anti-apoptotic effect of the p38 MAPK inhibitor, SB203580, on cells' apoptosis.

Results: Antimicrobial peptide could promote the proliferation, migration, and osteogenic ability of MC3T3-E1 cells induced by TNF-α, but inhibit cell apoptosis rate ($P<0.05$), and the effect was concentration-dependent. Western blot results showed after TNF-αtreatment, the expression of p-p38 MAPK in the MC3T3-E1 cells increased after TNF-α and antimicrobial peptide cotreatment, TNF-α induced p-p38 MAPK phosphorylation was inhibited, and the difference was statistically significant ($P<0.05$). Realtime PCR results showed that the gene expression of caspase-3 mRNA was up-regulated after TNF-α treatment, while their expression was down-regulated after cultured with TNF-α and antimicrobial peptide. Elisa's analysis showed that cell apoptosis increased after TNF-α treatment alone, and cell apoptosis was reduced to the normal levels when combined with antimicrobial peptide, and cell apoptosis induced by TNF-α was partially abolished when combined with SB203580.

Conclusions: Antimicrobial peptide DP7 could inhibit MC3T3-E1 cells apoptosis induced by TNF-α, and the effect was concentration-dependent. The antiapoptosis activation of the antimicrobial peptide on MC3TE-E1 cells may be related to the inhibition of the p38 MAPK pathway.

Keywords: Antibacterial peptides; MC3T3-E1 cells; apoptosis; p38 MAPK pathway

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Introduction

Periodontitis and peri-implantitis are chronic infectious diseases caused by pathogenic microorganisms. The bacteria, inflammatory factors produced by bacterial metabolism and their inducing immune response may lead to alveolar bone resorption and even implant failures (1). Except for periodontal scaling and root planning procedure, local usage of antibiotics can effectively inhibit bacteria and reduce the inflammation response. Unfortunately, the susceptibility to drug-resistance of antibiotics and the inflammatory cytokines released from dead bacteria will aggravate the inflammatory response (2). Current therapies could not thoroughly control the development of inflammation and recover the damaged alveolar bone tissue. How to prevent alveolar bone loss and increase the amount of regenerated bone has been an important research topic.

Alveolar bone is considered as a major supporting structure of periodontal tissues. And osteoblasts are the important functional cells for bone formation, which is involved in early osteogenesis and late bone reconstruction (3). Lipopolysaccharide (LPS) produced by dead bacteria and its inducing inflammatory cytokines (4), such as tumor necrosis factor-a (TNF-a) and interleukin-1 play a significant role in alveolar bone resorption, and they can increase osteoclasts production and promote osteoblast apoptosis (5,6). Studies have shown that LPS could affect the proliferation and apoptosis of osteoblasts through multiple signaling pathways transductions, such as the induction of proliferation through Notch pathway, the inhibition of apoptosis through JNK signaling pathway (7,8). P38MAPK signaling pathway is also shown to participate in regulating the apoptosis of osteoblasts induced by LPS (9).

Antimicrobial peptides (AMP) is a small cationic peptide with broad-spectrum antibacterial activity and minor drug-resistance, have definite clinical value in inhibiting pathogenic microorganisms and cancer cells (10,11). Also, antimicrobial peptides can inhibit the production of inflammatory factors, including TNF-α or IL-1, and promote tissue healing in damaged areas (12). In recent years, antimicrobial peptides have gradually attracted widespread attention of clinical scholars, and the development of new antimicrobial drugs has become a research hot spot.

Recent research has found that antimicrobial peptides could promote the differentiation, migration, proliferation of mesenchymal stem cells, also inhibit the formation of osteoclasts induced by LPS (13,14). It has also been reported that antimicrobial peptides enhanced the osteogenic differentiation of bone marrow mesenchymal stem cells by activating the bone morphogenetic protein-2/smad axis (15). However, the effect of antimicrobial peptides on the bioactivity of osteoblasts in the inflammatory environment and its related mechanism is still rarely reported. In this study, MC3T3-E1 cells induced by TNF-α were used to simulate the inflammatory condition, and the role of antibacterial peptide DP7 in inhibiting cell apoptosis through the p38 MAPK signaling pathway was investigated, which will provide the theoretical basis for its use in the treatment of periodontitis and peri-implantitis.

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-20-5338).

Methods

Main reagents and equipment

Mouse MC3T3-E1 cells line (Chinese Academy of Medical Sciences), antimicrobial peptides DP7 (State Key Biological Laboratory of Sichuan University), minimum essential medium (Gibco, America), alizarin red (Shanghai Sinopharm Group Co. LTD), TNF-α (Peprotech), Annexin V-FITC/PI Apoptosis Detection Kit (Miltenyi, Germany), CellTiter96® Aqueous One Solution Cell Proliferation Assay Kit (Promega), Real-time PCR Assay Kit (TaKaRa), Anti-phospho-MAPK (Cell signaling, America), BCA Protein Assay Kit (Beyotime). Inverted microscope (Olympus, Japan), Real-time PCR Detection System (Bio-Rad, America), fluorescence microscopy (Nikon, Japan), Gel electrophoresis system (Bio-Rad).

Study methods

Culture of MC3T3-E1 cells

MC3T3-E1 cells were cultured in a CO_2 incubator with MEM medium holding 10% fetal bovine serum,100 U/mL penicillin, and 100 mg/L streptomycin solution. The medium was changed every three days. When the cells were confluent to 70–80%, they were trypsinized using trypsin and subcultured.

Cell proliferation assay

MC3T3-E1 cells were seeded into 96-well plates at a density of 5×10^3 cells/well and divided into five groups:

MEM (A), 50 ng/mL TNF-α (B), TNF-α + 10 μg/mL AMP (C), TNF-α + 20 μg/mL AMP (D) and 10 μg/mL AMP (E). After 24 hours of incubation, TNF-α was added into groups b, c, and d at a final concentration of 50 ng/mL. Twenty-four hours later, AMP at a final concentration of 10 or 20 μg/mL were added into group C, E, or D. After another 24 h of incubation, 20 μL Cell Titer 96® Aqueous One Solution Reagent was added to each well and incubated for 2 h in the incubator. Then the absorbance of the supernatant was measured at 490 nm using a spectrophotometer, n>6. After incubation for 24 h, cells were stained by Hoechst 33342 dye and counted in five random fields on each sample using a fluorescence microscope (Leica).

Cell apoptosis assay

Cells were seeded into 6-well plates at a density of 1×10^4 cells/well and divided into four groups: MEM (A), 50 ng/mL TNF-α (B), TNF-α + 10 μg/mL AMP (C), TNF-α + 20 μg/mL AMP (D), which were treated as described in experiment 1.2.2. After 24 h of incubation, cells were trypsinized with trypsin, washed three times with phosphate buffer saline (PBS), then stained with Annexin-V labeled by FITC and PI for 15 min under the conditions. Within one hour, the fluorescence intensity of the early and late apoptotic cells were measured by using flow cytometry.

Detection of cell migration ability

Cells were seeded, grouped, and treated as described in experiment 1.2.3. After 24 h of culture, longitudinal scratches were made with a pipette in the center of well. At 1, 2, and 3 days after scratching, cell migration distance was respectively observed under the microscope, and images were taken at five random fields of view on each sample.

Mineralized nodule staining assay

Cells were seeded at 5×10^5 cells/well in a 24-well plate and treated as described in experiment 1.2.3. After incubation for 21 days, extracellular matrix (ECM) mineralization by the cells on the samples was assessed by Alizarin Red staining and observed using under a microscope. The images were taken at five random fields of view on each sample.

Alkaline phosphatase activity assay

Cells were seeded, grouped, and treated as described in experiment 1.2.3, with five duplicate wells per group. After culturing for 14 days, cells were washed with PBS three times and treated with 100 μL 0.2% TritonX-100. Then 50 μL of the above liquid was mixed with 50 μL PNPP substrate solution (4.5 mmol/L). After incubation at 37 ℃ for another 30 min, 50 μL NaOH (0.1 mol/L) was added to stop the reaction. The absorbance of the supernatant was measured at 490 nm.

Western blot detection of p-p38 MAPK protein expression

Cells were seeded, grouped, and treated as described in experiment 1.2.3. The protein in MC3T3-E1 cells was extracted with the lysate, placed on ice for 40 min, and centrifuged at 12,000 r/min for 40 min. Then the protein taken from the supernatant was quantified by the BCA method. Twenty μg protein samples were conducted sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane. The polyvinylidene difluoride(PVDF) membrane was put into the blocking solution for 1 h (37 ℃), diluted with the primary antibody, and incubated at 4 ℃ overnight. After being washed with PBS 3 times, the horseradish peroxidase marker was added to incubate with a diluted secondary antibody, and the membrane was shaken at 37 ℃ for 1 h. Enhanced chemiluminescence (ECL) exposure imaging is scanned into the computer.

The mRNA and protein expression of caspase-3

Cells were seeded, grouped, and treated as described in experiment 1.2.3. The primer was designed and synthesized with premier 5. MiniBEST Universal RNA Extraction Kit extracted total RNA and reverse-transcribed into cDNA according to the instructions of the Prime Script RT Master Mix Kit (TaKaRa). PCR reaction system included 1 μL of cDNA, 0.5 μL of upstream and downstream primers, 10 μL of SYBR Premix Ex Taq. After the reaction finished, the Mx 3000P real-time PCR system was used for quantitative analysis. Results were standardized according to the housekeeper gene and compared with the control group. Western blot detection of caspase-3 protein expression was same as the above experiment.

Elisa detection of cell apoptosis

Cells were seeded into 24-well plates at a density of 1×10^4 cells/well and divided into four groups: MEM, TNF-α, TNF-α + SB203580, TNF-α +AMP, TNF-α + AMP + SB203580. Cells were inoculated with AMP for 24 h and SB203580 for 3 h and then treated with TNF-α for 48 h. After the supernatant was removed, cells were treated with 0.5 mL Lysis Buffer for 30 min at room temperature

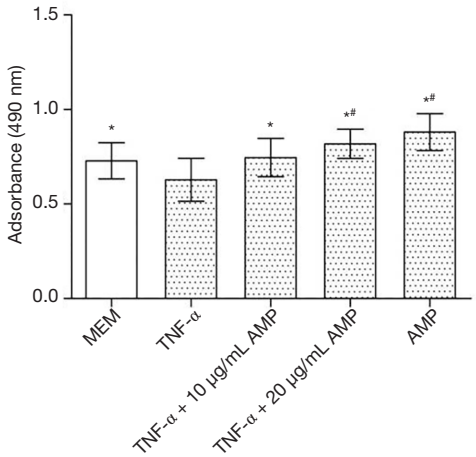

Figure 1 Effect of antibacterial peptides on the proliferation of MC3T3-E1 cells induced by TNF-α after 24 hours of incubation measured by MTS assay. *, P<0.05 compared to the treatment of TNF-α; #, P<0.05 compared to the treatment of MEM.

and centrifuged at 1,500 rpm for 10 min. Then 20 μL of supernatant and 80 μL immunoreagent (holding anti-histone and anti-DNA) were transferred into a streptavidin-coated microplate. After microplate was washed three times with incubation buffer, 100 μL of ABTS solution was added and incubated for 15 min. At last, the absorbance of the supernatant was determined at 405 nm.

Statistical analysis

SPSS 22.0 software was used for data analysis. The measurement data were expressed as ($\bar{x} \pm s$) and analyzed using SPSS 22.0 software. The statistical analyses were performed using A one-way analysis of variance (ANOVA), followed by an LSD (Least Significant Difference) test. A P value <0.05 was considered statistically significant.

Results

AMP promoted the proliferation of the MC3T3-E1 cells induced by TNF-α

The results of the MTS assay showed that the proliferation activity of the MC3T3-E1 cells induced by TNF-α decreased significantly, while the proliferation of the cells treated with antibacterial peptides increased (P<0.05). And when the concentration of AMP went higher, the cell proliferation rate increased more obviously (*Figure 1*). The fluorescent staining results showed in *Figure 2* also was consistent with that of MTS assay.

AMP inhibited the apoptosis of the MC3T3-E1 cells induced by TNF-α

The results of fluorescent dye detected by flow cytometry (*Figure 3*) showed that when treated with TNF-α, the early and late apoptosis MC3T3-E1 cells increased compared to the control group (P<0.05). When treated with AMP, the early and late apoptosis rate was inhibited, but the different concentrations of antibacterial peptides changed without significance.

AMP enhanced the migration ability of MC3T3-E1 cells induced by TNF-α

As shown in *Figure 4*, the migration ability of MC3T3-E1 cells treated with antibacterial peptides was higher than that treated with MEM or TNF-α, and the effect increased as the concentration increased. The migration ability of MC3T3-E1 cells induced by TNF-α was the slowest of all.

AMP promoted the osteogenesis of the MC3T3-E1 cells induced by TNF-α

As shown in *Figure 5*, the formation of the mineralized nodule of cells treated with antibacterial peptides was the most, which was proportional to the concentration of antibacterial peptides. Cells induced by TNF-α formatted the least mineralized nodule. As shown in *Figure 6*, the alkaline phosphatase of the cells induced by TNF-α had the lowest absorbance compared with the MEM or AMP group, and the difference is statistically significant (P<0.05). There was no significant difference between the AMP groups.

AMP inhibited p38 MAPK phosphorylation in MC3T3-E1 cells induced by TNF-α

The results of Western blotting showed that when treated with TNF-α, the expression of p-p38 MAPK in MC3T3-E1 cells was significantly up-regulated compared with other groups, and the difference was statistically significant (P<0.05). After treatment with antimicrobial peptide, the phosphorylation of p-p38 MAPK was partially inhibited, whose degree negatively correlated to the concentration of AMP. But the phosphorylation of the p-JNK protein and the p-ERK protein had no significant changes (*Figures 7,8*).

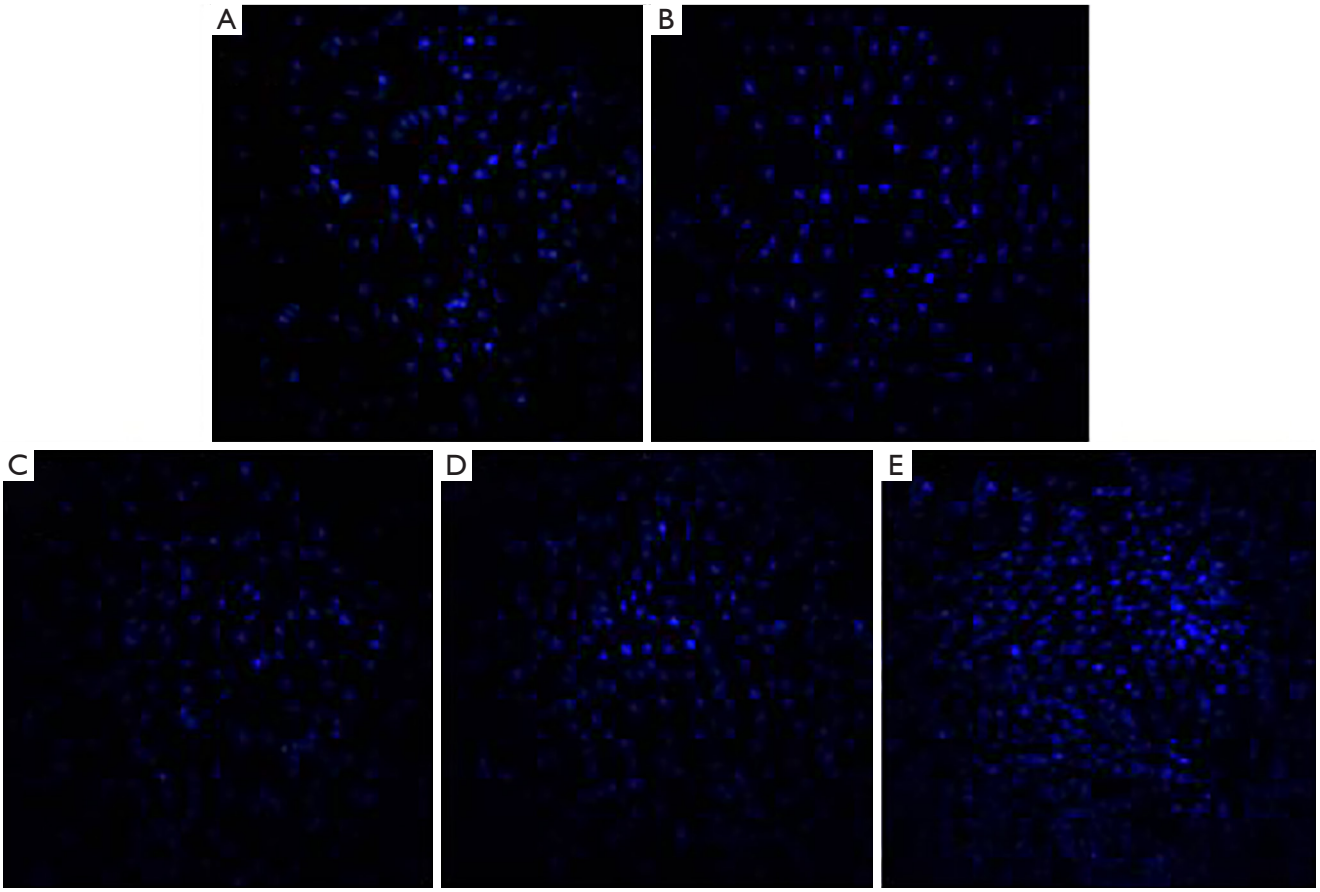

Figure 2 MC3T3-E1 cells stained by Hoechst33342 with fluorescence microscopy after 24 hours of incubation. (A) MEM group, (B) TNF-α treatment, (C) TNF-α and 10 μg/mL AMP, (D) TNF-α and 20 μg/mL AMP, (E) 10 μg/mL AMP.

AMP inhibited caspase-3 mRNA expression in MC3T3-E1 cells induced by TNF-α

Real-time PCR analysis (*Figure 9*) showed that after being treated by TNF-α, the expression of caspase-3 mRNA in MC3T3-E1 cells was up-regulated. However, after antibacterial peptide treatment, caspase-3 mRNA expression was down-regulated, with significant statistical difference compared with the control group (P<0.05).As shown in *Figure 10*, the caspase-3 protein expression of MC3T3-E1 cells treated with TNF-α increased, but when treated with AMP DP7,the protein expression of caspase-3 reduced.

AMP inhibited MC3T3-E1 cells apoptosis induced by TNF-α via p38 MAPK pathway

As shown in *Figure 11*, TNF-α treatment significantly increased MC3T3-E1 cells apoptosis, which had significant differences in statistics (P<0.05). When treated with a combination with SB203580 (a specific inhibitor of p38 MAPK), the apoptosis rate of MC3T3-E1 cells was inhibited to some extent, and there was a significant difference when compared with TNF-α treated group (P<0.05). After treated with SB203580, cell apoptosis rate in AMP treated group was back to normal, but there was no significant difference in cell apoptosis between two groups (P>0.05)

Discussion

Periodontitis is a common oral inflammatory disease, one of the leading causes of periodontal pockets, progressive attachment loss, and frontal resorption, finally leading to the loosening of teeth and loss of the tooth. It has a high prevalence and become an essential public health problem endangering people's health. Also, peri-implant

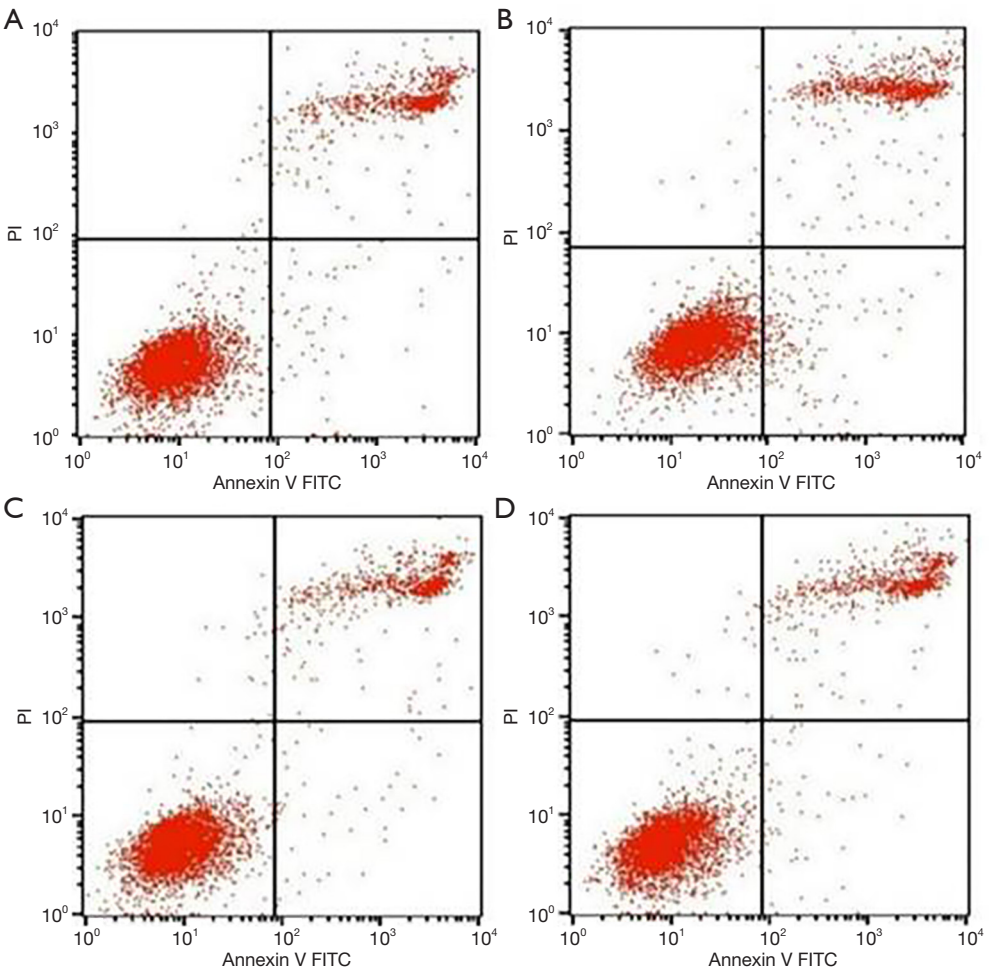

Figure 3 The apoptotic percentage of MC3T3-E1 cells after 24 hours of incubation measured by using Annexin V-FITC/PI Kit. Viable cells (annexin V−/PI−), early apoptotic cells (annexin V+/PI−), late apoptotic cells (annexin V+/PI+), and mechanical death cells (annexin V−/PI+) are located respectively in the bottom left, bottom right, top right, and top left quadrants. (A) Treatment with MEM; (B) treatment with TNF-α; (C) treatment with TNF-α and 10 μg/mL AMP; (D) treatment with TNF-α and 20 μg/mL AMP.

inflammation still is one of the major factors leading to implant failure. It is reported that five years after implantation, the incidence of peri-implant inflammation is as high as 14%. In addition to mechanical treatment, antibiotics are usually used to treat periodontal disease and peri-implant inflammation. However, the adverse reaction, dysbacteriosis, and resistance limited its clinical effectiveness. After antibiotics killed bacteria, substantial amounts of LPS were released, which stimulated macrophages to produce a series of inflammatory factors, including tumor necrosis factor, interleukin 6. The generation of osteoclasts and activation of osteoclasts process will be promoted directly or indirectly by inflammation factors.

Osteoblasts are the most critical functional cells for bone formation. Their proliferation and differentiation activity are the keys to osteogenesis, while their apoptosis rate increases bone resorption. The dynamic balance between osteoblasts and osteoclasts supports the relative stability of bone mass (16).

TNF-α, as important proinflammatory cytokines of periodontitis and periimplantitis, is one of the important pathogenic factors for bone resorption. It can inhibit osteoblast regeneration, reduce proliferation activity, and promote apoptosis (17). Several signaling pathways associated with inflammatory responses, including p38

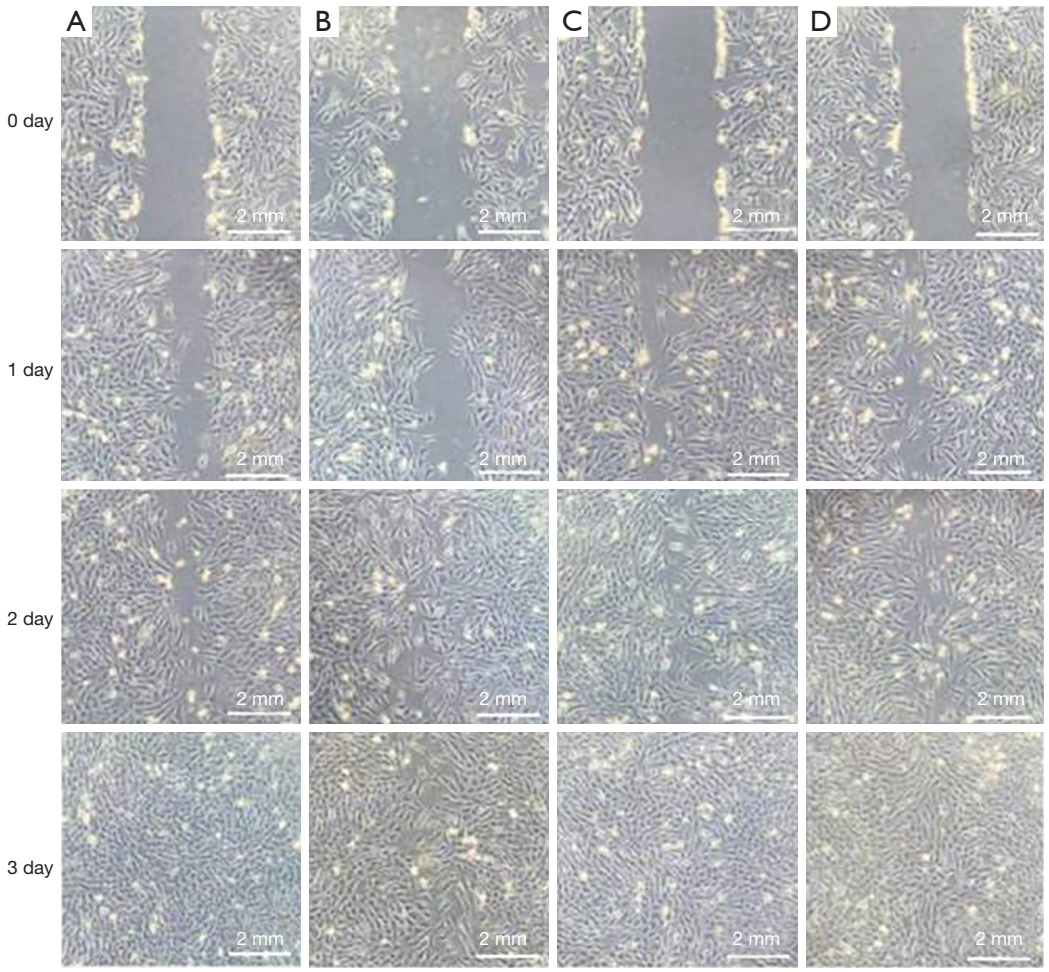

Figure 4 Migration of MC3T3-E1 cells after 1, 2, and 3 days of incubation. (A) Treatment with MEM; (B) treatment with TNF-α; (C) treatment with TNF-α and 10 μg/mL AMP; (D) treatment with TNF-α and 20 μg/mL AMP.

MAPK, Wnt, NF-κB may, play some role in the apoptosis of osteoblasts induced by TNF-α. It is accepted that when the concentration of TNF-α was higher than 50 ng/mL, it would significantly promote cell apoptosis (18). In this study, TNF-α induced apoptosis of osteoblasts was used as an inflammatory model.

Antibacterial peptides are the first defensive line of the natural immune system and widely found in animals and plants (19). Due to their unique antibacterial mechanism, they can inhibit viruses, bacteria, fungi, protozoa, and cancer cells but have no cleavage effects on healthy mammalian cells. As one of the most promising antimicrobial drugs, antimicrobial peptides are expected to become a substitute for antibiotics (20).

DP7 is a novel 12-amino-acid cationic and hydrophilic antimicrobial peptide with the broad antibacterial spectrum and minor drug-resistance. It has a strong ability of immune regulation and inhibition of TNF-α production from LPS (21). It was reported that when the concentration of AMP was 10–20 μg/mL, it had an excellent bactericidal effect and biological activity (22).

In recent years, antibacterial peptides are shown to have the potential to promote osteogenesis of osteoblasts. Some scholars (23) found that antimicrobial peptides can markedly promote the differentiation, migration, and proliferation of mesenchymal stem cells and inhibit the formation of osteoclasts induced by LPS. Tripathi (24) reported that self-assembled antibacterial peptide KLD-12 not only had adequate antibacterial activity but also can promote rapid tissue healing. Bacitracin is also shown to promote osteogenic differentiation of human bone marrow mesenchymal stem cells by stimulating the bone

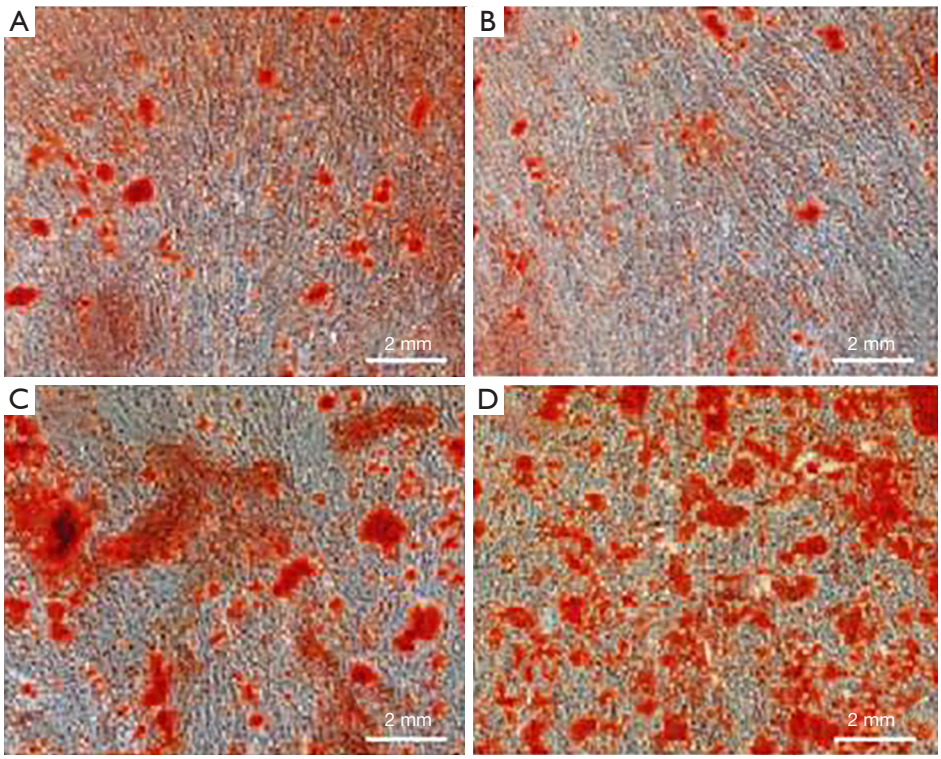

Figure 5 Mineralized nodule of MC3T3-E1 cells stained by Alizarin red S after 21 days of incubation. (A) Treatment with MEM; (B) treatment with TNF-α; (C) treatment with TNF-α and 10 μg/mL AMP; (D) treatment with TNF-α and 20 μg/mL AMP.

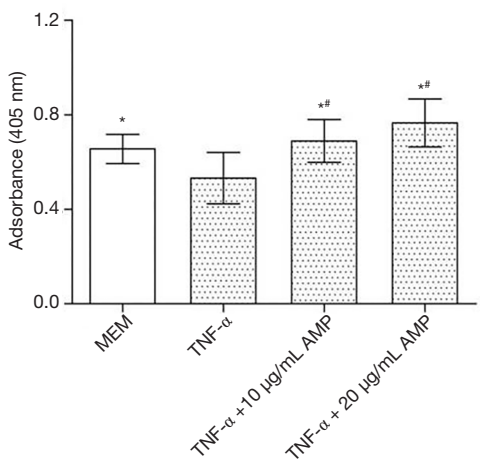

Figure 6 Alkaline phosphatase activity of MC3T3-E1 cells after 14 days of incubation. *, P<0.05 compared to the treatment of TNF-α; #, P<0.05 compared to the treatment of MEM.

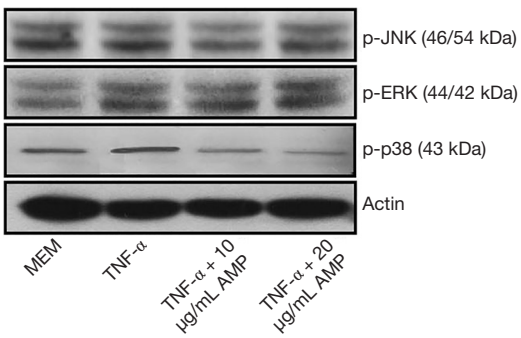

Figure 7 p-JNK, p-ERK and p-p38 MAPK protein expression of MC3T3-E1 cells after 24 hours of incubation detected by western blotting.

morphogenetic protein-2/Smad axis (25). Another recent study suggested that cationic antibacterial peptide P15-CSP has a unique dual ability to inhibiting the formation of biofilms and increasing osteogenic activity as a hydrophilic surface coating (26).

In this study, it was found that TNF-α increased apoptosis activity MC3T3-E1 cells and decreased their migratory ability, which showed that the inflammation model was successful. AMP could inhibit the apoptosis

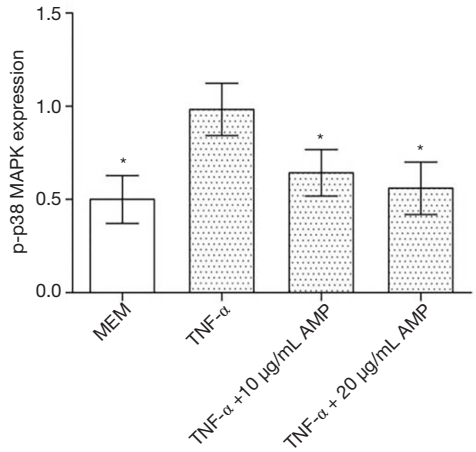

Figure 8 The p-p38 MAPK protein expression level of MC3T3-E1 cells after 24 hours of incubation detected by western blotting. *, P<0.05 compared to the treatment of TNF-α.

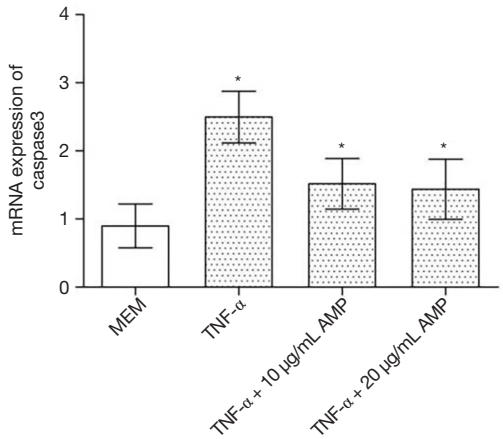

Figure 9 Caspase-3 mRNA level of MC3T3-E1 cells after 24 hours of incubation detected by Real-Time PCR. *, P<0.05 compared to the treatment of MEM.

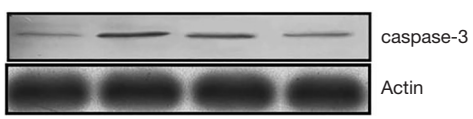

Figure 10 Western blotting showing caspase-3 protein expression of MC3T3-E1 cells after 24 hours of incubation detected by western blotting.

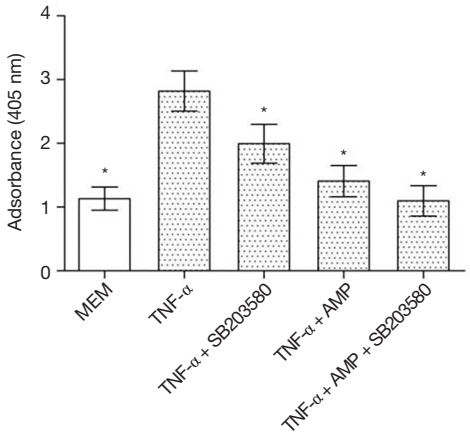

Figure 11 Cell apoptosis of MC3T3-E1 cells alone or combined with SB203580 detected by Elisa. *, P<0.05 compared to the treatment of TNF-α.

of MC3T3-E1 cells induced by TNF-α, and they increased with the increase of concentration. Bone tissue reconstruction depends on the migration of osteoblasts. Cell migration determination suggested TNF-α treatment decreased the migratory ability of osteoblasts and thus affected bone regeneration speed. ALP activity is an early marker of osteogenic differentiation in osteoprogenitor cells and is expressed after seven days of osteogenic induction, with positive staining after osteogenic induction for 14 days (27). Mineralized nodule formation is a phenotypic marker for the last stage of mature osteoblasts. The results showed that the higher the concentration of AMP was, the stronger was the promotion of osteogenic differentiation of MC3T3-E1 cells.

To further explore the possible mechanism, we studied the role of the MAPK pathway in the inhibition of antimicrobial peptides on TNF-α induced osteoblasts apoptosis. MAPK includes p38, JNK, and ERK1/2 pathways, which play an essential role in maintaining cell morphology, proliferation, differentiation, and apoptosis. Studies showed that the p38 MAPK pathway participates in regulating the growth, development, apoptosis, and skeleton construction of mesenchymal stem cells or osteoblasts (28). Some proinflammatory cytokines were reported to activate the p38 MAPK pathway. They might be related to the suppression of p38 MAPK activation (29), which was consistent with our experimental results. Western blot showed that TNF-α activated the phosphorylation of p38

MAPK pathway in MC3T3-E1 cells, while the activation of JNK and ERK signaling pathway did not change appreciably. Moreover, antimicrobial peptides played a part in the antiapoptosis of osteoblasts by the inhibition of p38 MAPK activation to some extent.

We also found SB203580, a specific inhibitor of p38 MAPK, partially inhibited apoptosis induced by TNF-8 but did not eliminate apoptosis of osteoblasts induced by TNF-i. Therefore, we concluded that antibacterial peptides inhibit TNF-n induced apoptosis of osteoblasts via the p38 MAPK signal pathway, and there may be other mechanisms adjusting the antiapoptosis activity of antibacterial peptides on MC3T3-E1 cells. Some scholars concluded that ERK signaling pathway is involved in the proliferation, differentiation in and apoptosis of osteoblasts (30). The inhibition of ERK pathway could suppress cytochrome C release from the mitochondrion and caspase-3 activation by adjusting the down-regulated expression of Bax and up-regulated expression of Bcl-2, which led to apoptosis of osteoblasts (31,32). It is also reported that activated mitogen-activated protein kinase could transfer into the nuclear via activating NF-κB, and regulate a variety of downstream inflammatory factors, which resulted of inflammatory reaction.

There are several signaling pathways transductions mediating cell apoptosis, such as death receptor pathway, mitochondrial pathway and endoplasmic reticulum-associated pathway, among which mitochondrial pathway is the most classical one. Caspase-3 is a critical protease in apoptosis signaling pathway and plays an essential pivotal role (33). Endogenous and exogenous apoptotic signals lead to cell apoptosis by activating caspase-3 (34). In this study, we found TNF-α induced the activation of caspase-3 in osteoblasts, but antimicrobial peptides suppress the activity of caspase-3, which suggested the antiapoptosis effect of antimicrobial peptides was related to the inhibition of caspase-3 activation. After p38 MAPK was activated, nuclear translocation took place, which could also phosphorylate many protein kinases and transcription factors. Therefore, the p38 MAPK pathway may play a role in both the upstream and downstream of the caspase.

Conclusions

From the above analysis, antimicrobial peptides DP7 could inhibit the apoptosis of MC3T3-E1 cells induced by TNF-αand showed some dose-effect relationship. Its inhibiting function might be due to reducing the phosphorylation of p38 MAPK signaling pathway and thus inhibiting caspase-3 activation, but its specific mechanism needed to be further explored.

Acknowledgments

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Footnote

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Data Sharing Statement: Available at http://dx.doi.org/10.21037/atm-20-5338

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-5338). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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