The Effects of Clindamycin and Cefazolin on Osteogenesis of Periodontal Ligament Stem Cells: An In vitro Study

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Introduction: Antibiotics are routinely administered in clinical setting for prevention of infections in surgeries and as a routine supplement to the culture medium. The nobility of the present study was to evaluate the effect of two commonly used antibiotics, clindamycin and cefazolin, in dental treatments, on osteogenic capability of periodontal ligament stem cells (PDLSCs). **Materials and Methods:** PDLSCs were isolated from periodontal ligament (PDL) of the root section out of four healthy extracted teeth. The cells were incubated in the following medium for 28 days: (1) Osteogenic medium (OM) + 5 µm clindamycin (clindamycin group), (2) OM + 5 µm cefazolin (cefazolin group) and (3) OM (control group). Alizarin red staining was performed at days 7, 14, 21, and 28 of induction. **Results:** Addition of cefazolin to osteogenic medium had no significant effect on osteogenesis comparing to the control group. However, Clindamycin significantly inhibited osteogenesis at days 7 and 21 comparing to the control group ($P<0.05$), but there was no significant differences at 14 and 28 days. **Conclusion:** It is demonstrated that clindamycin had inhibitory effects on osteogenesis in early stages, but it promoted osteogenesis in later stages. Cefazolin seems not inhibit osteogenic potential of the cells. Taken together, prescribing cefazolin or clindamycin did not cause any negative effect on osteogenesis for long-term.

Keywords: Antibiotics; Osteogenesis; Mesenchymal stem cells; Clindamycin; Cefazolin

Introduction

Annually 1.5 million people suffer from fracture subsequent to bone diseases (1) and it is estimated that 46% of US adults suffer from periodontal diseases (2). It is well-established that infections inhibit bone healing (3). Also, in periodontitis, persistent inflammation leads to tissue destruction and interferes with tissue healing and regeneration (4). Antibiotics are routinely administered for treatment or prevention of infection in orthopedic, (5), craniomaxillofacial traumas, and surgeries (6, 7). Moreover, antibiotics such as clindamycin and cefazolin are commonly prescribed as a non-surgical approach in treatment of periodontal diseases indeed (8). Bone repairs have shown to be affected by the number of osteoprogenitor cells; however, osteogenic potential for cells are quite crucial which could be influenced by antibiotic administration (9). Systemic administration of antibiotics has demonstrated to enhance regeneration in clinic (10); in contrast, several studies have indicated that antibiotics inhibit cell viability and function (11-15).

Cefazolin and clindamycin were routinely administrated for management of infection as well as prophylactic measures particularly when administration of penicillin provoked allergy reaction. However, there has been a controversy regarding the effect of these antibiotics on osteogenic regeneration as well as osteoprogenitor viability. It was demonstrated that administration of cefazolin precipitated a decrease in osteoblast growth and eventually leaded to cell death (16). According to the recent reports, the dosage of clindamycin showed no toxic effect at low doses; on the other hand, higher doses increased bone formation (17).

Periodontal ligament stem cells (PDLSCs) are multipotent stem cells within the periodontal ligament (PDL) with the capability to differentiate into the cementoblasts, osteoblasts, and fibroblasts (18, 19). Due to the pivotal role of PDLSCs in periodontium regeneration the purpose of the present work was to assess the effect of clindamycin and cefazolin, in periodontal disease on osteogenic potential of PDLSCs.

Materials and Methods

PDLSCs were isolated from roots of four healthy premolars which were extracted due to orthodontic purposes. A week prior to extraction right before extraction, scalling and root planning were performed in the entire teeth. The extraction was performed

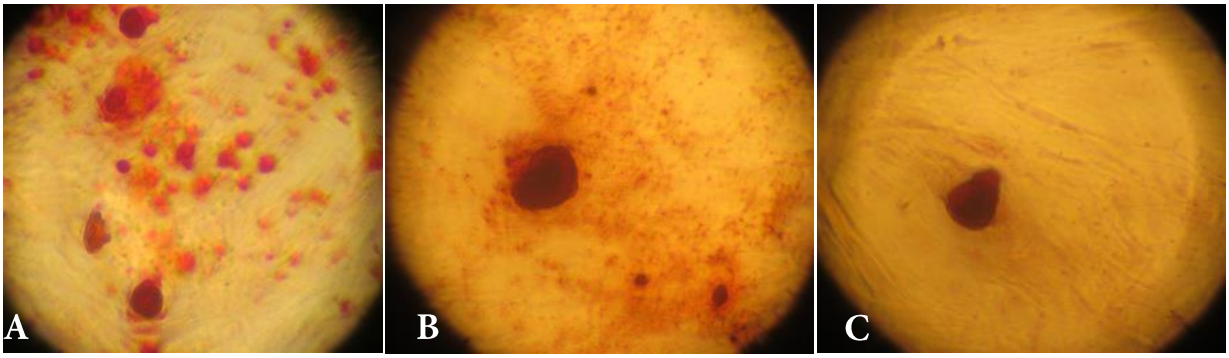

Figure 1. Results of Alizarin red staining in 7th day (A) Control; (B) Cefazolin; (C) Clindamycin

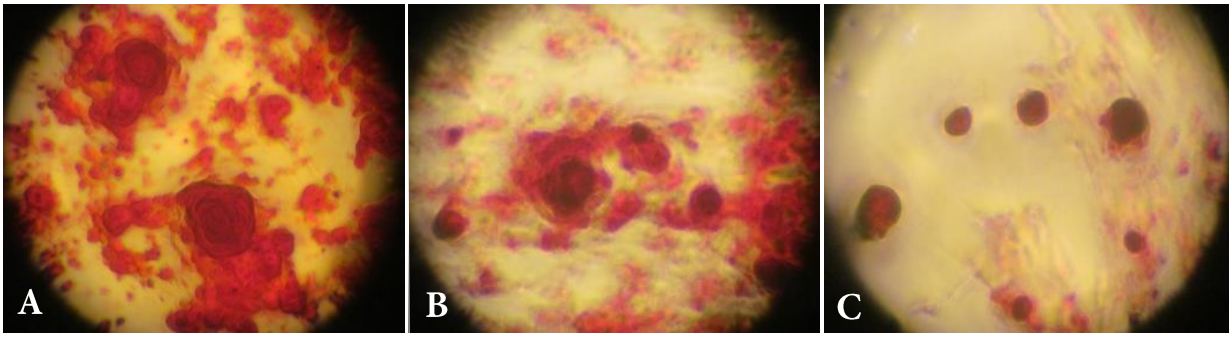

Figure 2. Results of Alizarin red staining in 14th day (A) Control; (B) Cefazolin; (C) Clindamycin

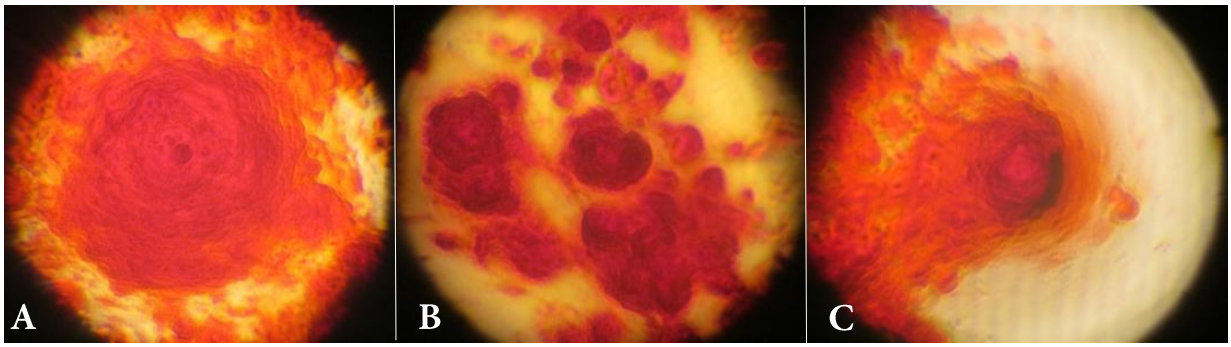

Figure 3. Results of Alizarin red staining in 21th day (A) Control; (B) Cefazolin; (C) Clindamycin

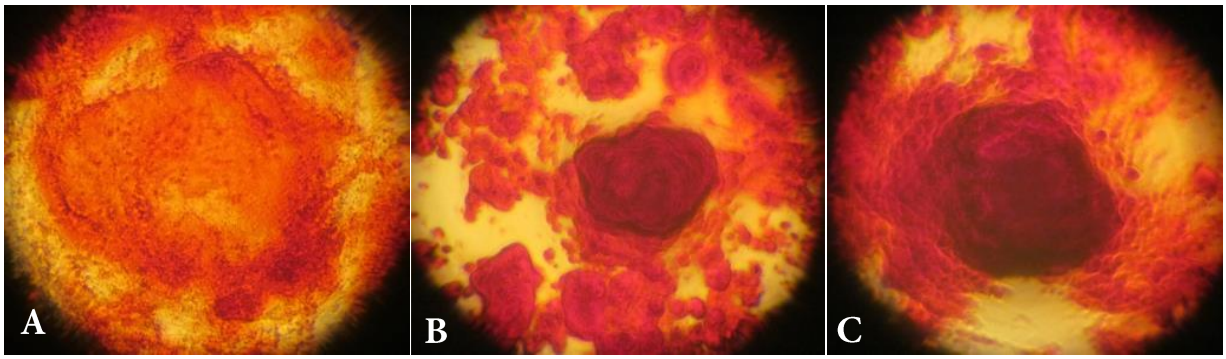

Figure 4. Results of Alizarin Red Staining in 28th day (A) Control; (B) Cefazolin; (C) Clindamycin

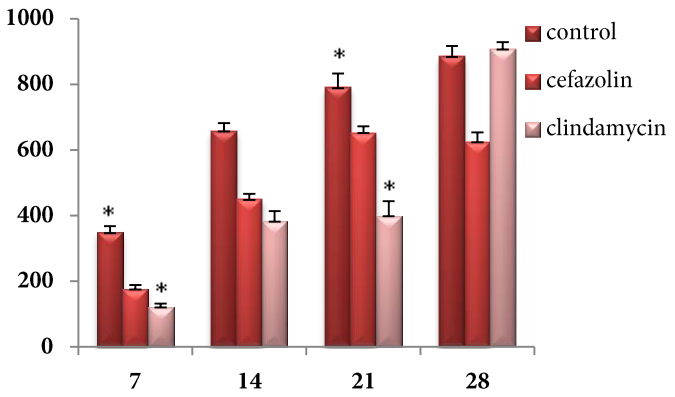

Figure 5. Quantified alizarin red staining results among three study groups. (* indicates significant difference among designated groups at $P \leq 0.05$)

according to the following steps: prior to the extraction, the patients rinsed their mouth with diluted povidine iodine (Kaveh, Iran). Then, the extraction procedure was performed under sterile conditions. Next, the crown and root were separated by a disk under copious irrigation and the roots were transferred into a tube containing α-MEM (Gibco, Paisley, UK); eventually, the PDL was separated by a surgical blade. The tissue from the middle portion of the root surface was utilized for cell isolation and the tissue was rinsed with phosphate buffered saline (PBS, Gibco, Paisley, UK) and centrifuged at 400g for 10 minutes.; then it treated with 1 mL type I collagenase (3 mg/mL) and 1 mL dispase II (4 mg/mL) for 1 h at 37˚C. Then, the solution was removed and washed with PBS. The pellet PDL cells were transferred to culture medium containing α-MEM, 15% fetal bovine serum (FBS), and 1% penicillin-streptomycin. Upon 80% confluency, cells were passaged by using 0.25% trypsin-EDTA (Gibco, Paisley, UK). Cells at passage 3 were transferred into osteogenic medium (OM) containing DMEM, 50 μg/mL ascorbic 2-phosphate (Sigma Aldrich, St. Louis, MO, USA), 10 nmol/L dexamethasone (Sigma Aldrich, St. Louis, MO, USA), and 10 mmol/L β-glycerol phosphate (Sigma Aldrich, St. Louis, MO, USA) lacking penicillin streptomycin; then they were allocated into three different treatment groups as follow: (1) OM + 5 μm clindamycin (clindamycin group); (2) OM + 5 μm cefazolin (cefazolin group); (3) OM only (control). The cells were cultured in given conditions for 28 days. In each week the osteogenic differentiation of the cells were evaluated by 5 minutes staining with 40 mmol/L Alizarin red staining, pH 4.2m.

Results

Alizarin red staining results demonstrated that clindamycin group had less osteogenic efficacy compare to cefazolin (P=0.017) in the first week of induction (Figure 1). However, it promoted osteogenesis after 28 days. Its osteogenic effect was comparable with the other two groups (P-values were P=0.98 and 0.14 for cefazolin and control, respectively) (Figure 5).

Cefazolin enhanced osteogenesis from early stages and it was reached the plateau after 3 weeks with a pattern similar to the control group (P>0.05) (Figure 3A-C). At day 28th, no significant difference was observed among groups (P>0.05) (Figure 4A-C, Figure 5).

Discussion

Routinely, cefazolin and clindamycin are administrated for the management of infection as well as prophylactic measures. particularly when administration of penicillin causes allergic reaction (20) . Also, they are an integral part of cell culture in laboratory (21). Knowledge of antibiotics effects would better assist clinicians for adjusting the dosage while minimizing the side effects (9). The literature reviews has demonstrated controversial results regarding the effect of antibiotics for *in vitro* osteogenesis (10-15). In this study, PDLSCs were obtained from PDLs of healthy teeth followed by treatment with osteogenic medium supplemented with cefazolin or clindamycin for 28 days. We particularly showed that cefazolin dosage does not affect PDLSC osteogenesis while clindamycin affects it in early stages, while in long term it dosage does not hamper osteogenesis.

Comparison of administration of different antibiotics, cefazolin, showed more toxic effect on cell growth compared to vancomycin. It was observed that cefazolin inhibited cell growth at concentration of 200 μg/mL and osteoblasts were died when exposed to 10,000 μg/ml cefazolin, while higher dosage of vancomycin did not affect cell proliferation rate (16). Therefore, clindamycin has cytotoxic effects at 400 μg/ml dosage (22). On the contrary, loading cefazolin on biodegradable polypeptide multilayer nanofilms on orthopedic implants which improved viability and proliferation of osteoblasts (23). The controversy between the results in the current and previous reports may be attributed to higher doses of antibiotic administered in their study. Due to variations in cell lines used; as well as, incubation time and drug dosage among different studies, we were not able to reach a general assumption regarding the effect of cephalosporines on osteogenesis.

It is suggested that clindamycin inhibits osteoblast differentiation at concentration of 10 μg/ml. Also, it is cytotoxic at concentrations of 500 μg/ml and above (17). This finding may describe the delayed stimulating effect of clindamycin on PDLSCs; although we could verify this assumption by performing cell viability assays. It is demonstrated that 10 μg/ml clindamycin positively affects ALP activity in 24 and 48 hrs, while concentrations greater than 500 μg/ml significantly decreased ALP activity (17). The controversy regarding our findings and this study may be attributed to differences in incubation time and various doses of drugs which may drastically affect the outcomes.

Conclusion

Addition of clindamycin to osteogenic medium has inhibitory effects on osteogenesis in early stages, however, its effect in longer incubation time is comparable to cultures lacking antibiotics. Cefazolin does not seem to have any significant effect on osteogenesis.

Conflict of Interest: 'None declared'.

References

1. General S. Bone health and osteoporosis: a report of the surgeon general.
2. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. J Periodontol. 2015;86(5):611-22.
3. Loi F, Córdova LA, Pajarinen J, Lin T-h, Yao Z, Goodman SB. Inflammation, fracture and bone repair. Bone. 2016;86:119-30.
4. Thomas MV, Puleo DA. Infection, inflammation, and bone regeneration: a paradoxical relationship. J Dent Res. 2011;90(9):1052-61.
5. Pountos I, Georgouli T, Bird H, Kontakis G, Giannoudis PV. The effect of antibiotics on bone healing: current evidence. Expert opinion on drug safety. 2011;10(6):935-45.
6. Andreasen JO, Jensen SS, Schwartz O, Hillerup Y. A systematic review of prophylactic antibiotics in the surgical treatment of maxillofacial fractures. J Oral Maxillofac Surg. 2006;64(11):1664-8.
7. Mueller SC, Henkel KO, Neumann J, Hehl EM, Gundlach KK, Drewelow B. Perioperative antibiotic prophylaxis in maxillofacial surgery: penetration of clindamycin into various tissues. J Craniomaxillofac Surg. 1999;27(3):172-6.
8. Santos CMML, Lira-Junior R, Fischer RG, Santos APP, Oliveira BH. Systemic Antibiotics in Periodontal Treatment of Diabetic Patients: A Systematic Review. PloS one. 2015;10(12):e0145262.
9. Rathbone CR, Cross JD, Brown KV, Murray CK, Wenke JC. Effect of various concentrations of antibiotics on osteogenic cell viability and activity. J Orthop Res. 2011;29(7):1070-4.
10. Nowzari H, Matian F, Slots J. Periodontal pathogens on polytetrafluoroethylene membrane for guided tissue regeneration inhibit healing. J Clin Periodontol. 1995;22(6):469-74.
11. Ince A, Schutze N, Karl N, Lohr JF, Eulert J. Gentamicin negatively influenced osteogenic function in vitro. Int Orthop. 2007;31(2):223-8.
12. Zhou X, Zhang P, Zhang C, An B, Zhu Z. Tetracyclines inhibit rat osteoclast formation and activity in vitro and affect bone turnover in young rats in vivo. Calcif Tissue Int. 2010;86(2):163-71.
13. Cheng W, Yue Y, Fan W, Hu Y, Wang X, Pan X, et al. Effects of tetracyclines on bones: an ambiguous question needs to be clarified. Pharmazie. 2012;67(5):457-9.
14. Chang Y, Goldberg VM, Caplan AI. Toxic effects of gentamicin on marrow-derived human mesenchymal stem cells. Clin Orthop Relat Res. 2006;452:242-9.
15. Kallala R, Graham SM, Nikkhah D, Kyrkos M, Heliotis M, Mantalaris A, et al. In vitro and in vivo effects of antibiotics on bone cell metabolism and fracture healing. Expert Opin Drug Saf. 2012;11(1):15-32.
16. Edin ML, Miclau T, Lester GE, Lindsey RW, Dahners LE. Effect of cefazolin and vancomycin on osteoblasts in vitro. Clin Orthop Relat Res. 1996(333):245-51.
17. Naal FD, Salzmann GM, von Knoch F, Tuebel J, Diehl P, Gradinger R, et al. The effects of clindamycin on human osteoblasts in vitro. Arch Orthop Trauma Surg. 2008;128(3):317-23.
18. Dalband M, Amiri I, Soltanian AR, Gholami M, Khayati A, Keshmirypor V. Comparing Viability of Periodontal Ligament Stem Cells Isolated From Erupted and Impacted Tooth Root. J Craniofac Surg. 2015;26(7):e608-12.
19. Houshmand B, Behnia H, Khoshzaban A, Morad G, Behrouzi G, Dashti SG, et al. Osteoblastic differentiation of human stem cells derived from bone marrow and periodontal ligament under the effect of enamel matrix derivative and transforming growth factor-beta. Int J Oral Maxillofac Implants. 2013;28(6):e440-50.
20. Addy L, Martin M. Clindamycin and dentistry. British dental journal. 2005;199(1):23-6.
21. Perlman D. Use of antibiotics in cell culture media. Methods in enzymology. 1979;58:110-6.
22. Duewelhenke N, Krut O, Eysel P. Influence on mitochondria and cytotoxicity of different antibiotics administered in high concentrations on primary human osteoblasts and cell lines. Antimicrob Agents Chemother. 2007;51(1):54-63.
23. Li H, Ogle H, Jiang B, Hagar M, Li B. Cefazolin embedded biodegradable polypeptide nanofilms promising for infection prevention: a preliminary study on cell responses. J Orthop Res. 2010;28(8):992-9.

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