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 novelty 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 (cefa 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 lead 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, scaling 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
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.

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).
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
