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Immunoprecipitation high performance liquid chromatographic analysis of healing process in chronic suppurative osteomyelitis of the jaw

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ABSTRACT

Purpose: Chronic suppurative osteomyelitis (CSO) of the jaw is one of the most difficult infectious diseases to manage, because it causes progressive bony destruction and is associated with bacterial inhabitation of the sequestra. A combination of antibiotic therapy and surgical debridement is often used to treat CSO. Nevertheless, various systemic conditions can lead to life-threatening complications.
Methods: The present study aimed to explore the wound healing progress in 16 cases of CSO through protein expression analysis of postoperative exudates (POE) that were collected 6 h, 1 day, and 2 days after saucerization and/or decortication. A bony lesion was removed during surgery and then examined pathologically, and the CSO POE was examined by immunoprecipitation thus high performance chromatography (IP-HPLC). The POE at 6 h was used as a comparative control.
Results: Histologically the CSO lesion showed a necrotic granulomatous lesion heavily infiltrated with polymorphonuclear leukocytes, macrophages, and plasma cells, admixed with multiple sequestra inhabited by bacterial colonies. The IP-HPLC analysis displayed a slight increase in innate immunity-related proteins, i.e., NFkB, TNFα, IL-1, IL-6, IL-28, and LL-37, but a gradual decrease of bacteria-related inflammatory proteins, i.e., IL-8, IL-12, CD31, CD68, and lysozyme. The angiogenesis-related proteins, i.e., VEGF-A and VEGF-C, were slightly decreased but TGF-β1 and bFGF were markedly increased on day 2. The osteogenesis-related proteins, i.e., OPG and ALP, were slightly increased, while the osteoclastogenesis-related protein, RANKL was slightly decreased compared to the control.
Conclusion: These findings indicate that the infected CSO undergoes a rapid wound healing process with active osteogenesis and a gradual decrease in bacteria-related inflammation, predicting a favorable prognosis after surgery. Moreover, IP-HPLC can be useful in monitoring the POE and wound healing processes during the postoperative period.

1. Introduction

Jaw osteomyelitis is a serious disease caused by oral infection. It most often affects elderly people who have various systemic illnesses (Krakowiak, 2011; Lieblich and Piecuch, 2000; Nary Filho et al., 2014) (Fig. 1). Specifically, in some cases of severe chronic suppurative osteomyelitis (CSO) of the jaw, the postoperative prognosis is unfavorable even after intensive antibiotic therapy or is unknown even when considering individual patient risk factors (Armstrong and Rush, 1983; Chen et al., 2013). Moreover, the life-threatening complications of CSO are exacerbated when combined with debilitating systemic diseases, e.g., diabetes or HIV, or chemotherapy or radiation therapy for oral cancer (Sudjaritruk et al., 2012; Huh et al., 2015; Peravali et al., 2012; Marx and Tursun, 2012; Chrcanovic et al., 2010).

CSO of the maxillofacial region is primarily caused by infections of symbiotic oral microorganisms. It may also arise as a

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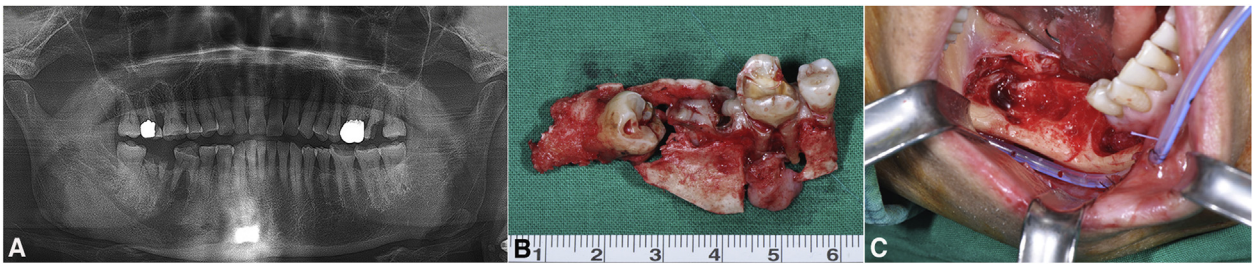

Fig. 1. Clinical and radiological findings of case number 16, showing a preoperative panoramic view (A), surgical specimens (B), and the intraoral wound with drain insertion (C).

complication of dental extractions, maxillofacial trauma, inadequate treatment of a fracture or bone graft, and irradiation to the mandible (Nary Filho et al., 2014; Kumar et al., 2013; Yadav et al., 2014; Rajkumar et al., 2010). Actinomycotic osteomyelitis is relatively common because these bacteria are normal commensals, but *Actinomyces* becomes a pathogen when it gains entry into tissue layers and bone and establishes and maintains an anaerobic environment with extensive sclerosis and fibrosis. This infection spreads contiguously, frequently ignoring tissue planes and surrounding tissues and organs (Gannepalli et al., 2015).

Although recent advances in antimicrobial therapy have generally been successful in treating CSO, the heterogeneous bacteria of CSO are able to evolve against different antibiotics. Therefore, the identification of bacterial strains in a CSO infection is essential; cultures can be collected during a radical sequestrectomy and the debridement of the involved area for the treatment of CSO (Lore et al., 2013). The surgical method of saucerization and decortication first appeared in the literature in 1946 (McClintock, 1946a, McKelvey, 1946b, McClintock, 1946c). Thereafter, many authors recommended decortication and saucerization for the treatment of CSO (Shannon et al., 1973; Kim and Jang, 2001). These surgeries can provide continuous postoperative drainage of inflammatory exudate from the high-pressure area within the marrow space covered by cortical bone to the low-pressure area of the cancelous bony surface. However, many surgeons expect that postoperative antibiotic therapy will be successful, and prefer to close the saucerization surface as soon as possible in order to prevent the secondary infection; a drainage tube is usually inserted to remove exudate from the edematous postoperative lesion. In this case, it is assumed that some inflammatory exudate including pathogenic bacteria will be retained, resorbed, and phagocytized by the immune system. This inflammatory exudate might remain after the closure of saucerization and cause a delay of wound healing and a recurrence of CSO. Therefore, in this study, a decompressed drainage apparatus was used to remove postoperative exudate (POE) from the surgical wound (Fig. 2A).

The present study included 16 cases of CSO and assessed the POE obtained from the saucerization or decortication wounds of the CSO. The POEs were safely collected through a decompressed drainage apparatus, which was made to remove the POE actively via mild negative pressure of a peristaltic pump (Fig. 2B). Subsequently the POEs were analyzed using immunoprecipitation thus high performance liquid chromatography (IP-HPLC) to quantify protein expression relevant to the wound healing progresses of a CSO lesion (Fig. 2C–E). The results of IP-HPLC analysis are compared to what is known about the clinical course of healing during the postoperative period, and the clinical implications are also discussed.

2. Materials and methods

A total of 16 cases of CSO were included from the medical records of the Department of Oral and Maxillofacial Surgery at Seoul National University Dental Hospital between September 2015 and

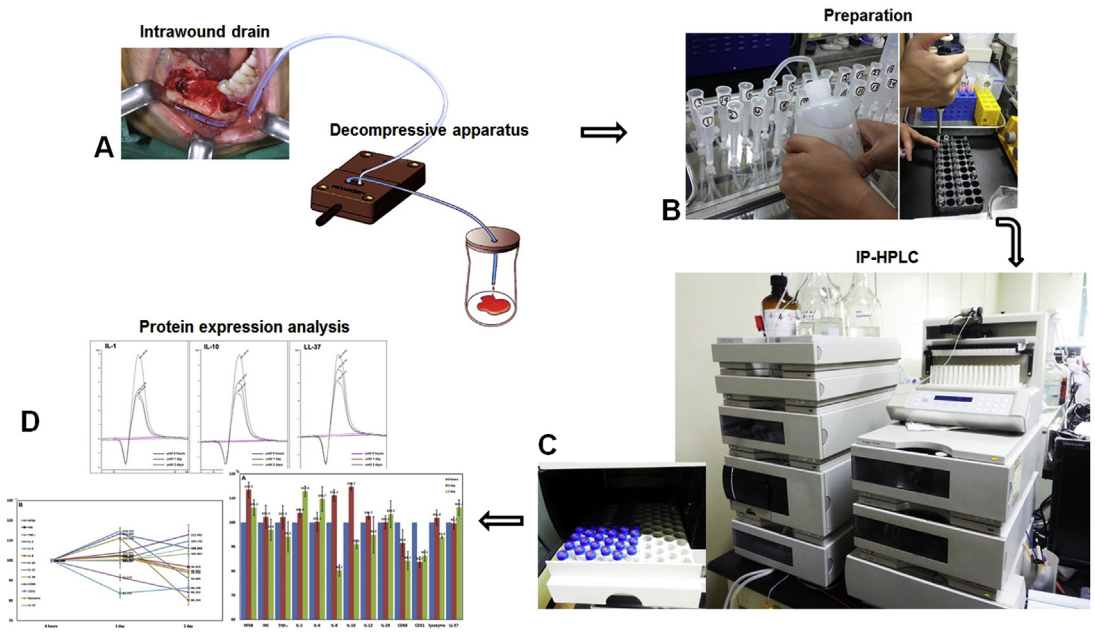

Fig. 2. The whole sequence of procedures of this research, from the surgical wound drain to the final protein expression analysis through IP-HPLC methods.

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