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Effects of systemic administration of sitafloxacin on subgingival microflora and antimicrobial susceptibility profile in acute periodontal lesions

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ABSTRACT

The aim of this study was to assess the effect(s) of systemic administration of sitafloxacin on subgingival microbial profiles of acute periodontal lesions. Antimicrobial susceptibility of clinical isolates was also investigated. Patients with acute phases of chronic periodontitis were subjected to clinical examination and microbiological assessment of their subgingival plaque samples by culture technique. Sitafloxacin was then administered (100 mg/day for 5 days) systemically. The clinical and microbiological examinations were repeated 6–8 days after administration. Susceptibilities of clinical isolates to various antimicrobials were determined using the broth and agar dilution methods. From the sampled sites in 30 participants, a total of 355 clinical isolates (34 different bacterial species) were isolated and identified. *Parvimonas micra*, *Prevotella intermedia* and *Streptococcus mitis* were the most prevalent cultivable bacteria in acute sites. Systemic administration of sitafloxacin yielded a significant improvement in clinical and microbiological parameters. Among the antimicrobials tested, sitafloxacin was the most potent against the clinical isolates with an MIC_{90} of 0.12 μg/ml at baseline. After administration, most clinical isolates were still highly susceptible to sitafloxacin although some increase in MICs was observed. The results suggest that systemic administration of sitafloxacin is effective against subgingival bacteria isolated from acute periodontal lesions.

1. Introduction

Periodontitis is a plaque biofilm-induced inflammatory disease of the supporting tissue of the tooth. In acute phase of periodontitis, an expressed breakdown of periodontal tissue occurs during a limited period of time, with easily detectable clinical symptoms [1,2]. Since it is associated with substantial morbidity and presents a possible risk of infection spreading, an urgent care is needed. Failure to treat appropriately may result in progressive loss of periodontal attachment affecting an adverse change in prognosis and possible tooth loss [3].

For the antimicrobial treatment of acute periodontal lesions, β-lactam antibiotics still remain to be the drug of first choice because of their broad-spectrum antimicrobial effects. However, β-lactamase activity has been detected in subgingival sites of subjects with chronic periodontitis at levels capable of inactivating β-lactam antibiotics passing into periodontal pockets through gingival crevicular fluid [4]. Recently, it was reported that β-lactamase-positive subgingival bacterial species were detected in more than one-half of subjects with severe chronic periodontitis [5]. Moreover, β-lactam antibiotics were shown to possess low effect against selected strains of *Fusobacterium nucleatum*, which plays an important role in the organization of polymicrobial biofilm [6].

In recent years, azithromycin has been used as a unique adjunctive agent in the management of periodontitis. Although azithromycin has a similar spectrum of antibacterial activity to erythromycin, it appears to have significantly improved potency against Gram-negative organisms [7]. It has also been shown to be effective against biofilms containing *Porphyromonas gingivalis*, one of the most prominent periodontal pathogen [8,9]. While the use of azithromycin can be an effective adjunctive modality in the

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treatment of periodontitis, a concern has been raised regarding the increased incidence of bacterial resistance to macrolide antibiotics. One study demonstrated that *F. nucleatum*, *Parvinomonas micra* and *Prevotella intermedia* showed high MIC values for azithromycin [10].

New quinolones have been shown to be effective against various anaerobes [11] and *Aggregatibacter actinomycetemcomitans* [12], a pathogen associated with aggressive periodontitis. However, their antimicrobial activities against other periodontal pathogens such as *P. gingivalis*, *F. nucleatum*, *P. intermedia* and *Tannerella forsythia* were reported to be less than optimal [13]. Sitafloxacin is an oral fluoroquinolone antimicrobial agent with broad-spectrum antibacterial activity against Gram-positive and Gram-negative aerobes and anaerobes [14–16]. Since June 2008, this drug has been used clinically in Japan for a number of conditions including pneumonia, cystitis, and pyelonephritis [17].

Using whole genomic DNA probes and checkerboard DNA–DNA hybridization, Socransky and co-workers classified periodontal microbial communities using a color-coded system that reflected cluster analysis, community ordination and associated disease severity [18]. Among these groups, the so-called 'red complex' is a group of three species, including *P. gingivalis, Treponema denticola* and *T. forsythia*, which are strongly associated with each other and with diseased sites [18]. In a previous study from our research group, sitafloxacin showed *in vitro* high antimicrobial activity against periodontal pathogens including the red complex bacteria and *F. nucleatum* [6]. In a clinical study, systemic administration of sitafloxacin was shown to be effective at improving periodontal health of elderly during supportive periodontal therapy [19]. These findings lead us to consider that sitafloxacin can be effectively used in periodontal chemotherapy.

Periodontal pathogens such as *P. gingivalis* have been reported to form a sizeable proportion of microorganisms from periodontal abscesses [1,20,21]. However, information is still limited regarding the microbial profiles and antimicrobial treatment strategy of acute periodontal lesions. The aim of this study was to assess the effect(s) of systemic administration of sitafloxacin on subgingival cultivable microflora of acute periodontal lesions. Antimicrobial susceptibilities of clinical isolates were also determined before and after administration.

2. Materials and methods

2.1. Participants

Study participants were recruited from patients who visited the Conservative Dentistry, Tokyo Dental College Chiba Hospital (Chiba, Japan) or Dentistry and Oral Surgery, Keio University Hospital (Tokyo) during the period of March 2012 through May 2013. Patients were asked to participate if they were diagnosed with acute phase of chronic periodontitis [22]. Exclusion criteria included uncontrolled systemic diseases, history of allergic reaction to antimicrobial agents, history of antimicrobial or anti-inflammatory therapy in the previous 6 months, <30 years of age, pregnant or lactating women. A total of 31 Japanese patients with acute phase of chronic periodontitis were recruited in the present study.

All participants provided a written informed consent. Ethical approvals for this study were obtained from institutional review boards of Tokyo Dental College (No. 322) and Keio University School of Medicine (No.2011-239), and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

2.2. Clinical examinations

After collection of full medical and dental histories, the signs and symptoms of spontaneous pain, percussion pain, pain caused by pressure, occlusal pain, swelling, redness, and suppuration in the inflamed area were recorded. Periodontal parameters including probing depth (PD), bleeding on probing (BOP) and tooth mobility (TM) [23] were also recorded. PD was measured using a Williams probe with an approximate force of 0.25 N and rounding to the nearest millimeter. BOP was recorded as the presence or absence of bleeding after measurement of PD.

2.3. Microbiological assessment of subgingival plaque samples

For subgingival plaque sampling, one acute site in each patient was chosen as the experimental site (A1), and one chronic site (C1) as the control site. Criteria for the acute phase included at least two of the following clinical signs or symptoms; pain, swelling, redness or a feeling of warmth in the periodontal lesion [2]. In case of pronounced periodontal abscess that might require drainage through external incision or tooth extraction, such patient was excluded. For chronic (control) site, an effort was made to choose site with comparable PD with acute site.

Subgingival plaque samples were collected by inserting a sterile paper point (Absorbent Paper Points, size 040, Zipperer, Munich, Germany) for 10 s into the deepest area of the pocket accessible after carefully removing the supragingival plaque with sterilized cotton pellets. The paper point was placed into a transport tube (Seed Tube, Eiken Chemical, Tokyo) for the microbiological assessment by culture method.

2.4. Intervention

After sampling, gentle subgingival irrigation with sterile saline solution was performed in both experimental and control sites. No mechanical subgingival debridement was performed at this time. Thereafter, systemic sitafloxacin (Gracevit®, Daiichi Sankyo, Tokyo) was administered (100 mg/day for 5 days). Compliance was monitored by completion of a daily checklist. Clinical and microbiological examinations of acute sites and control sites were repeated at 6–8 days after the sitafloxacin administration (designated as A2 and C2).

Subsequently, the study participants received further periodontal therapy as needed.

2.5. Microbiological assessment

Microbiological samples were transported to a microbiology laboratory (Mitsubishi Chemical Medience, Tokyo) within 6 h at 4°C, homogenized with sterile physiological saline and then cultured on both selective and non-selective agar media for the detection of aerobic and anaerobic bacteria and testing for antimicrobial susceptibility. Bacteria on basal and selective media were identified by colony morphology, staining characteristics, biochemical tests, API system and VTEK 2 (Biomerieux SA, Marcy l'Etoile, France). All laboratory procedures were performed by personnel that were blinded to the clinical status of study participants, and their inclusion in the present analysis.

2.6. Antimicrobial susceptibility testing

Antimicrobial susceptibilities of clinical isolates to amoxicillin (Sigma–Aldrich, St. Louis, MO, USA), cefcapene (Shionogi, Osaka, Japan), cefdinir (Sigma–Aldrich), clarithromycin (Sigma–Aldrich), azithromycin (LKT Laboratories, St. Paul, MN), minocycline (Sigma–Aldrich), levofloxacin (Daiichi Sankyo) and sitafloxacin (Gracevit®, Daiichi-Sankyo) were determined. MICs for aerobic bacteria was determined according to a standard broth dilution method recommended by CLSI [24] using cation-adjusted Mueller Hinton