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ORIGINAL ARTICLE

Microbial changes in patients with acute periodontal abscess after treatment detected by PadoTest

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AIMS: To investigate changes in bacterial counts in subgingival plaque from patients with acute periodontal abscess by IAI-PadoTest.

MATERIALS AND METHODS: Ninety-one patients were randomly allocated to either test or control groups. In all the patients, pockets with acute periodontal abscess were irrigated with sterilized physiological saline, and in the test group, 2% minocycline hydrochloride ointment was applied once into the pocket in addition. Subgingival plaque samples were collected by paper point before treatment and 7 days after treatment. The total bacterial count was determined and *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, and*Tannerella forsythia*, were detected using IAI-PadoTest, a DNA/RNA probe method.

RESULTS: The total bacterial count decreased in both groups, with a significant decrease in the test group. The counts and number of sites positive for *P. gingivalis*, *T. forsythia* and *T. denticola* significantly decreased in the test group after treatment, compared with those in the control group. Pocket depth decreased in the both groups, with a statistically significant decrease in the test group.

CONCLUSION: Topical treatment with minocycline in pockets with acute periodontal abscess was effective in reducing the bacterial counts as shown by the microbiological investigation using PadoTest 4.5.

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Keywords: periodontopathic bacteria; acute periodontal abscess; PadoTest; DNA/RNA probe

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Introduction

Periodontitis is an inflammatory bone disease affecting tooth-supporting tissues caused by periodontopathic bacteria that proliferate in periodontal pockets. An acute periodontal abscess, which is a localized purulent inflammation of the periodontal tissues, is a frequent periodontal condition in which periodontal tissues may be rapidly destroyed. Little information is available regarding the diagnosis and microbiology of periodontal abscesses. This acute infection occurs in the walls of periodontal pockets as a result of the invasion of bacteria into the periodontal tissues. Periodontal abscesses are termed 'mixed anaerobic infections', based on microbiological findings. However, there have been only a few bacteriological reports and controversy exists regarding the treatment of this condition. A recent study reported that periodontal abscesses showed clinical features typical of untreated periodontitis, with periodontopathic bacteria mainly involved and use of antibiotic adjunctive therapy in abscess treatment may be rational (Jaramillo *et al*, 2005). Reduction of the bacterial load in the periodontal pocket by pocket irrigation or administration of a topical antibiotic before performing mechanical debridement might relieve the severe inflammation in periodontal abscesses. The effectiveness of this method based on bacterial changes has not been established so far. In the treatment of periodontitis, long-term administration of host modulating agents such as matrix metalloproteinase inhibitors, nonsteroidal anti-inflammatory drugs and biphosphonate anti-osteolytic agents have been shown to be effective in arresting the progression of periodontal disease (Reddy *et al*, 2003), but to manage acute phases of periodontitis, systemic or topical application of antimicrobial agents is generally performed in order to reduce the bacterial load rapidly.

Various methods have been employed to detect the presence of periodontopathic bacteria in pockets (Suido *et al*, 1988; Loeshe *et al*, 1990; Ishihara *et al*, 1992; Boyer *et al*, 1996; Boutaga *et al*, 2003; Tanaka *et al*,

2006). However, no method is appropriately simple, reliable and cost-effective to be applied as a bacterial test for monitoring changes during periodontal therapy. Recently, polymerase chain reaction (PCR) and DNA/DNA or DNA/RNA probe methods were developed for the detection of periodontopathic bacteria (Ashimoto *et al*, 1996; Conrads, 2002). Compared with bacterial culture, which is considered the gold standard, these microbial tests are less time consuming, easy to perform and inexpensive. Hence, these modern methods of molecular biology are frequently used for microbiological investigations nowadays (Beikler *et al*, 2004; Jervoe-Storm *et al*, 2005; Nonnenmacher *et al*, 2005; Dahlen and Leonhardt, 2006). The IAI-PadoTest 4.5 (IAI Inc., IAI Institute, Zuchwil, Switzerland) is a system for detecting periodontopathic bacteria using the DNA/RNA probe method. Few studies have employed this technique for detection and typing of bacteria in diagnosis and treatment of periodontitis (Kamma and Baehni, 2003; Mombelli *et al*, 2005). In the present study, IAI-PadoTest 4.5 and oligonucleotide probes were employed to detect periodontopathic bacteria in patients with acute periodontal abscess. The aim of the study was to evaluate bacteriological variations after different therapeutic methods for acute periodontal abscess using the IAI-PadoTest.

Materials and methods

Selection of subjects and test teeth

The study population consisted of 91 patients (30 males and 61 females, mean age 57.3 ± 10.4 years), who visited the dental clinics at Tokyo Medical and Dental University and Hokkaido University for emergency treatment of acute periodontal abscess at a single site in the oral cavity. Patients were excluded, if they had received any treatment or antibiotics for acute periodontal abscess 3 months prior to the study; had systemic diseases; or if they were judged to be ineligible by the chief clinical investigator or clinical investigators based on allergic history or psychological health. Smokers were not excluded from the study. The study protocol was explained clearly to all subjects, and those who gave informed consent were enrolled in the study. The experimental protocol had been approved by the ethics committees of both institutions.

The test tooth consisted of a single tooth, either single rooted or multi-rooted, affected by a periodontal abscess in each subject. All clinical parameters and bacterial sampling were performed in the same teeth throughout the experiment.

Clinical evaluation

The pocket depth (PD) and clinical attachment level (CAL) of the test teeth were measured in all subjects after plaque sampling. A periodontal probe was used to record the above clinical parameters at six sites of each tooth. Pain, inflammation, tooth mobility, and bleeding on probing were evaluated in all subjects at the same appointment for additional data.

Treatment

The subjects were randomly allocated to test and control groups, according to a computer-generated allocation table. The person-in-charge, who was not involved in the study, kept the allocation key code until the end of the study and thus the allocation was concealed. In the test group (46 patients: 15 males, 31 females), the periodontal pocket with the acute periodontal abscess was irrigated with sterilized physiological saline, and 2% minocycline hydrochloride ointment for dental use (Periocline® dental ointment; Sunstar Inc., Osaka, Japan) was administered once into the periodontal pocket. In the control group (45 patients: 15 males, 30 females), only irrigation of the periodontal pocket with 10 ml of sterilized physiological saline was performed.

Bacteriological investigation

Subgingival plaque samples were collected from periodontal pockets with an acute periodontal abscess on day 0 (before treatment) and on day 7 after treatment. Sampling was performed before probing and one sampling site nearest to the periodontal abscess was selected. Before sampling, supragingival plaque was wiped by sterilized cotton rolls and saliva around sampling region was gently dried with compressed air. A medium-sized paper point (Johnson & Johnson, Medical Inc., Arlington, TX, USA) was inserted into the deepest region of the periodontal pocket of the test tooth, left in place for about 10 s and then transferred to an attached tube containing 100 μl of 4 M guanidinium thiocyanate 2-mercaptoethanol and immediately stored at −80C until sample transport. All samples were transported by international express mail without temperature control. The microbial samples were sent to the IAI Inc., and counts of four main periodontal bacteria, *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, and the total bacterial count were measured, using IAI-PadoTest 4.5 standard procedures (Kamma and Baehni, 2003). In brief, the collected samples were mounted on nylon membranes and hybridized to ^{32}P-labeled specific probes directed against the small subunit ribosomal RNAs (ssrRNAs) of the previously mentioned four periodontal bacteria and a universal bacterial probe (UP) for total bacteria. Bacterial counts were calculated by comparison with homologous reference standards and expressed as counts of 10^6.

Statistical analysis

The statistical analysis was performed with the aid of a standard statistical computer program (SPSS version 11.0, SPSS Japan Inc., Tokyo, Japan). Total bacterial count, PD and CAL before and after treatment were compared using the *t*-test. A change in total bacterial count before and after treatment was compared using the chi-square test. The number of sites positive for the four bacterial strains before and after treatment (%) was compared using the Fisher's exact test. A *P*-value less than 0.05 was considered significant.

Table 1 Total bacterial count (TBC) before and after treatment

Bacterial count ($\times 10^6$)	*Day 0*	*Day 7*
T group ($n = 46$)	13.58 ± 12.85	4.40 ± 4.22 **
	NS	*
C group ($n = 45$)	14.43 ± 25.92	11.85 ± 23.81*

Data are shown as mean ± s.d.
t-test: NS, not significant; $^{**}P < 0.01$, $^{*}P < 0.05$.

Results

Changes in clinical parameters
The mean PD and CAL before treatment and changes in PD and CAL after treatment were evaluated. In the test group, the PD significantly decreased on day 7 (Δday 0–day 7: 0.56 ± 0.67) compared with the control group (Δday 0–day 7: 0.18 ± 0.55). No significant difference was observed in CAL.

Changes in total bacterial count and between-group comparison
Changes in the total bacterial count in the test (T group) and control (C group) groups are shown in Table 1. The total bacterial count was 13.58×10^6 on day 0 and 4.40×10^6 on day 7 in the T group ($P < 0.01$), and 14.43×10^6 on day 0 and 11.85×10^6 on day 7 in the C group ($P < 0.05$), showing a significant decrease from day 0 to day 7 in both groups. A between-group comparison showed that no significant difference was present on day 0, but the total bacterial count was significantly lower in the T group on day 7 ($P < 0.05$).

Detection rate of each bacterial species
Changes in the detection rate of each bacterial species before and after treatment are shown in Table 2. The detection rates were determined from the number of sites/total site (%) positive for the four bacteria. *Tannerella forsythia*, *P. gingivalis* and *T. denticola* were significantly lower on day 7 than on day 0 in the T group ($P < 0.01$). A comparison of the T and C groups on day 7 showed that the detection rates of *T. forsythia*, *P. gingivalis*, and *T. denticola* were significantly lower in the T group ($P < 0.01$). No significant differences were observed in the detection rate of *A. actinomycetemcomitans* both before and after treatment in each group and between the groups, probably because of the low detection frequency and bacterial count.

In addition, the number of patients in whom the total bacterial counts decreased after treatment was significantly higher in the T group.

Table 2 Detection rate of the four bacterial strains by using PadoTest before and after treatment (%)

	Group T ($n = 46$)		*Group C* ($n = 45$)	
	Day 0	*Day 7*	*Day 0*	*Day 7*
A. actinomycetemcomitans	10.9	0	2.2	6.7
T. forsythia	69.6	13.0	60.0	53.3
P. gingivalis	71.7	15.2	57.8	48.9
T. denticola	69.6	15.2	60.0	42.2

Fisher's exact test: $P < 0.01$ for *T. forsythia* (group T day 0 *vs* group T day 7, and group T day 7 *vs* group C day 7); *P. gingivalis* (group T day 0 *vs* group T day 7, and group T day 7 *vs* group C day 7); *T. denticola* (group T day 0 *vs* group T day 7, and group T day 7 *vs* group C day 7).

Discussion

Topical antibiotic administration for acute periodontal abscess was effective in reducing the PD and bacterial number in periodontal pockets without mechanical debridement. In contrast, pocket irrigation showed a moderate reduction in PD but there was no change in the total bacterial count.

Bacterial detection of periodontal pathogens has been performed by many methods including culture, PCR and DNA probes. The PCR and DNA probe methods have become popular and are commonly used these days for microbial detection. The greatest advantage of these DNA-based tests is that even relatively small amounts of bacteria can be detected. In the present study, we used oligonucleotide probe hybridization for quantification of the periodontal pathogens in periodontal abscesses. The IAI-PadoTest 4.5 is a system for detecting periodontopathic bacteria using the DNA/RNA probe method. This method employs an oligonucleotide probe technique for determining the total bacterial count and detecting the four main periodontal pathogens *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia*, and *T. denticola* (Kamma and Baehni, 2003). PadoTest 4.5 was compared with detection by monoclonal antibody immunofluorescence in nearly 500 sites. The two methods had an agreement of around 80% for *T. forsythia*, *P. gingivalis*, and *T. denticola* and 70% for *A. actinomycetemcomitans* (Geinoz *et al*, 1997). Studies reporting the specificity of oligonucleotide probe method showed that all oligonucleotide probes had species specificity by cross-reaction checks, except few cases between *P. gingivalis* and related species (Dix *et al*, 1990; Moncla *et al*, 1990). The PadoTest 4.5 has been used to evaluate the changes in microbial load in a few studies (Kamma and Baehni, 2003; Mombelli *et al*, 2005). In regions of acute periodontal abscess, bacteria in periodontal pockets are believed to grow rapidly and cause infection, leading to formation of abscesses. However, there have only been a few investigations of the bacterial dynamics in periodontal abscesses, and only a limited number of reports are available in the last 10 years.

Umeda *et al* (1996) reported an initial total bacterial count (before treatment) of 1.47×10^6 in periodontal pockets exhibiting acute symptoms, as measured by the culture method, with a decrease to 4.41×10^5 1 week after application of 2% minocycline ointment to the periodontal pockets. Consistent with this, Herrera *et al* (2000a) reported that the bacterial count in periodontal pockets, again measured by culture method, was 1.06×10^6 in patients with periodontal abscess, and Hafstrom *et al* (1994) reported that the total bacterial count in periodontal pockets with an abscess was of the

order of 10^6 upon initial examination, but decreased following 2-week administration of antibiotics, reaching orders of 10^5 and 10^4 42 and 180 days after treatment, respectively.

Tominaga (1994) simultaneously measured the total bacterial count using culture method and phase-contrast microscopy. The counts were 1.50×10^6 and 7.73×10^7, respectively, and those 1 week after application of 2% minocycline ointment were 3.70×10^5 and 1.79×10^7, respectively. Using IAI-PadoTest 4.5, the initial value (before treatment) in the present study was 1.36×10^7 in the T group and 1.44×10^7 in the C group, and these values decreased to 4.40×10^6 in the T group and 1.19×10^7 in the C group after 1 week, showing results similar to those measured by Tominaga using phase-contrast microscopy (Tominaga, 1994).

In the investigation of bacterial species, *P. gingivalis*, *T. forsythia* and *T. denticola* were detected in about 70% of all tested sites, suggesting that these three bacteria play an important role in the development of acute periodontal abscess as in chronic periodontitis. As *A. actinomycetemcomitans* was detected in only about 10% or less of the pockets examined, it was concluded that this bacterium has no direct relationship with the onset of acute periodontal abscess. In the C group, the detection rates of the three significant bacterial species did not significantly differ before and after treatment. In contrast, the counts of the three bacteria and the number of sites in which they were detected were significantly decreased in the T group after treatment, compared with treatment with irrigation alone (Table 2). Herrera *et al* (2000b) administered systemic antibiotics to patients with periodontal abscess for 3–8 days, and investigated changes in the bacterial flora. The changes observed in our study were comparable with or larger than those after systemic antibiotic administration, indicating the usefulness of local chemotherapy in single tooth with acute periodontal abscesses for which limited mechanical treatment is performed.

In reports investigating the clinical and bacteriological effects of local chemotherapeutic agents administered into periodontal pockets in patients with periodontitis (Murayama *et al*, 1991; Kinane and Radvar, 1999; Van Steenberghe *et al*, 1999), scaling and root planing (SRP) was concomitantly performed. It has been recommended that the periodontal biofilm needs to be mechanically disrupted prior to administration of chemotherapeutic agents for obtaining the best results in periodontal pockets. In our study, SRP was not performed because the treated sites had severe inflammation and accompanying pain due to the acute periodontal abscess. Furthermore, the effectiveness of topical treatment in reducing the inflammation prior to performing mechanical treatment was investigated. This approach may reduce the risk of bacteremia in periodontitis patients by reducing the initial bacterial load.

Specifically, the objective parameter, PD, was significantly decreased 7 days after treatment in the T group, compared with that in the C group. Bacteria in the periodontal pockets are likely to decrease rapidly upon treatment. This may result in improvement of local inflammation, in turn leading to a rapid reduction of PD. However, re-evaluation after a longer period may be necessary to confirm the effect of the treatment protocol.

A few reports have correlated changes in PD and bacterial composition at given sites (Simonson *et al*, 1988; Riviere *et al*, 1995; Takeuchi *et al*, 2001; Kawada *et al*, 2004; Yoshida *et al*, 2004). We organized changes in the count of each bacterial species at each site and changes in PD at each site into a matrix, and investigated the relationship between improvement in PD and changes in each bacterial species (data not shown). This analysis showed that changes in the counts of *P. gingivalis*, *T. forsythia*, and *T. denticola* were more consistent with changes in PD than with the total bacterial count, suggesting that the levels of these three bacterial species reflect the effect of treatment on PD.

PadoTest can be easily handled by dental surgeons and the subgingival plaque samples can be sent to the laboratory by post without any special preparation or under any specific condition. However, PadoTest uses probes of labeled ribosomal RNA and synthetic small DNA for detecting target bacteria, and it must be performed in a well-controlled and validated laboratory. So, it is recommended that every general practitioner should send the sample to the specific laboratory in Switzerland. Chair side tests provide important information which aids in formulating the treatment plan, but current chair side tests are only able to determine whether the samples are positive or negative for the target bacteria and the quantity of sample required for detection is limited. Although the PadoTest cannot be performed chair side, it is able to determine quantitatively even small amounts of bacteria in the test sample.

In conclusion, local treatment with minocycline in pockets with acute periodontal abscess was effective in reducing the PD and bacterial counts. Microbiological investigation using PadoTest 4.5 may give an accurate reflection of the clinical effect, suggesting that it is of value for determining bacterial changes in periodontal pockets with favorable treatment outcome.

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