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H.　知的財産件の出願・登録状況
該当なし

III. 研究成果の刊行に関する一覧表

別紙５

研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷り

Relationships of anti-PAc (361–386) peptide salivary IgA antibody, eosinophils and basophils with periodontal status in the elderly

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Abstract

The amino acid residues 361–386 of *Streptococcus mutans* PAc includes an important region associated with the interaction between *S. mutans* and salivary components. We investigated the relationships between levels of the anti-PAc (361–386) peptide antibody (PPA) in saliva and periodontal status in 281 elderly subjects (mean age 77 years; 118 females, 163 males) by assessing dental calculus (CA), attachment loss (AL), pocket depth (PD), bleeding on probing (BOP) and various blood parameters. Enzyme-linked immunosorbent assay results revealed that subjects with a PPA level of greater than 0.1 (PPA detected group) showed a lower average value for number of sites with more than 6 mm of AL/6 points × 100/tooth (rAL6) than those with a PPA level of less than 0.1 (PPA not detected group). Furthermore, average values for rAL6 were significantly lower in the PPA detected group, and BOP, AL and rAL6 correlated positively and significantly with the percentage of eosinophils present in leukocytes in female subjects in both groups. PPA level had a negative correlation with percentages of basophils and eosinophils. The results indicate that systemic increases in numbers of eosinophils and basophils are associated with the development of periodontal diseases, while PPA level may be a useful indicator of periodontal status.

Introduction

In Japan, the majority of elderly people suffer from periodontitis (Dental Health Division of Health Policy Bureau Ministry of Health and Welfare Japan, 1999), which is a major factor in tooth loss (Ong, 1998; Olver & Brown, 2000). However, at present there is no known useful indicator or predictor of periodontal status. Periodontal diseases are chronic inflammatory conditions that affect the well-vascularized connective tissues of the periodontium (Egelberg, 1996). At the end of the 1990s, aggressive periodontitis was redefined as a complex disease exhibiting microbial alterations and cellular dysfunction that is differentiated from chronic periodontal disease by the underlying molecular mechanisms of its pathogenesis (Armitage, 1999). Periodontal inflammation may have an effect on and worsen systemic conditions associated with leukocyte migration from the bloodstream into tissues at the site of inflammation, and several prominent neutrophils have been implicated as amplifiers of that inflammatory response (Batino *et al.*, 1999; Yamalik *et al.*, 2000).

In the human oral cavity, gram-negative anaerobic organisms reside in a complex mixed-species biofilm that forms on tooth surfaces and in periodontal pockets, with *Porphyromonas gingivalis*, a gram-negative anaerobe, recognized as one of the primary pathogens in severe manifestations of adult periodontitis (Socransky & Haffajee, 1992; Kolenbrander & London, 1993). Recently, Lamont *et al.* reported that the surface protein antigen of *Streptococcus gordonii* (SspB), which is also a member of the highly conserved PAc (Okahashi *et al.*, 1989) in *Streptococcus mutans*, interacted with fimbriae from *P. gingivalis*, as shown by the results of *in vitro* assays (Lamont *et al.*, 1994; Park *et al.*, 2005). *Streptococcus gordonii* and *Streptococcus sanguinis* are early colonizers of the salivary pellicle, while *S. mutans* colonizes at later stages. However, the abilities of each to bind to salivary proteins and glycoproteins as well as the differences in affinity to the salivary pellicle between *P. gingivalis* and *S. mutans* may be related to the virulence of supra- or subgingival microbial communities in dental diseases.

Streptococcus mutans has been reported to have an association with the development of biofilm and dental

caries on tooth surfaces (Hamada & Slade, 1980; Loesche, 1986). The function of the cell-surface protein antigen of *S. mutans*, also known as PAc (Okahashi *et al.*, 1993), Ag I/II (Russell & Lehner, 1978), PI (Forester *et al.*, 1983) and B (Russell, 1979), is essential for colonization by the bacterium on tooth surfaces as well as its interaction with the salivary pellicle that coats dental enamel (Russell & Mansson-Rahemtulla, 1989; Demuth *et al.*, 1990; Senpuku *et al.*, 1996a). The alanine-rich repeating region (residue 219–464, A-region) of the PAc molecule, which is important for bacterial interaction with the salivary pellicle (Brady *et al.*, 1992; Nakai *et al.*, 1993; Timmerman *et al.*, 2000), has a strong immunogenicity in humans (Senpuku *et al.*, 1996b) and has been proposed as a candidate antigen for inducing the production of antibodies that inhibit the adherence of *S. mutans* to tooth surfaces (Senpuku *et al.*, 1995, 2001). The PAc (361–377) peptide in the A-region containing the epitope has also been shown to induce an antibody that inhibits the interactions of *S. mutans* with salivary components on tooth surfaces and is considered to be important for the adherence of *S. mutans* to tooth surfaces (Senpuku *et al.*, 2001; Takeuchi *et al.*, 2001). The overlapped area of the PAc (370–386) peptide to the PAc (361–377) peptide includes a multiple binding motif (L––V–K––A) that reacts with human leukocyte antigen (HLA)-*DRB1*0802*, **1101*, **1402* and **1405* genotypes, and is also found in the A-region (Senpuku *et al.*, 1998). The high production of salivary IgA antibody levels in reaction to the coupled PAc (361–386) peptide from residues 361–377 and 370–386 was reported to be a unique indicator of population and proportion of mutans streptococci (mS), such as *S. mutans* and *Streptococcus sobrinus*, because low and high concentrations of the salivary antibody were found to be correlated positively and negatively, respectively, with the concentrations of mS in saliva from human subjects (Tsuha *et al.*, 2004).

In our search for a new indicator or predictor of periodontal diseases, the present study analysed the relationships between the production of anti-PAc (361–386) peptide salivary IgA antibody (PPA), blood status and periodontal status. PPA level was found to be associated with attachment loss (AL) in both males and females, and bleeding on probing (BOP) and pocket depth (PD), as well as concentrations of total streptococci (tS), lactobacilli (LB) and basophils in females. The results may provide important information on PPA for the development of preventive medicines for periodontal diseases.

Materials and methods

Human subjects

In 1998, a longitudinal interdisciplinary study of ageing was initiated to evaluate the relationships between health status and dental diseases, such as root caries and periodontal disease, in Japan. Initially, questionnaires were sent to all 4542 residents aged 70 years (born in 1927) in Niigata City. After dividing by sex, 600 subjects were selected randomly, with approximately the same numbers of each sex chosen for the baseline survey (Yamaga *et al.*, 2002; Yoshihara *et al.*, 2003). The participants agreed to undergo medical and dental examinations, and signed informed consent forms regarding the protocol, which was approved by the Ethics Committee of Niigata University Graduate School of Medical Dental Science. The study was carried out in accordance with the Helsinki Declarations. Follow-up surveys have been carried out every year in June using the same methods as in the baseline survey. Among the participants ($n = 399$) in the follow-up survey conducted in June 2005, 281 subjects (average age 77 years old; 118 females, 163 males) participated in the present measurements of salivary antibodies and blood parameters.

Dental examinations were conducted under artificial white light by trained dentists. According to WHO criteria (WHO, 1986), decayed teeth (DT), missing teeth (MT) and filled teeth (FT) (DMFT) scores were recorded along with findings of dental caries. Four calibrated dentists assessed subject periodontal conditions based on the results from six measurements points (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual) around each tooth. Intra- and interexaminer reliability was confirmed using a kappa statistic ($k = 0.56$–0.92 for AL). Two hundred fifty-eight subjects (112 females and 146 males) participated in the present measurements of periodontal status. To estimate periodontal status, rCA (rate of occurrence of sites with dental calculus), rAL4 (rate of occurrence of sites with greater than 4 mm of AL), rAL6 (6 mm of AL), rPD4 (rate of occurrence of sites with greater than 4 mm of PD), rPD6 (6 mm of PD), AL [length (mm) of attachment loss] and BOP (rate of occurrence of sites with blooding on probing) were also measured at the same six points of each tooth (Ogawa *et al.*, 2002). Thereafter, the indicators were assessed and used to estimate the periodontal status of each subject, according to the methods explained below.

Synthetic peptide

The sequences of PAc (361–386) (NAKATYEAALKQYEADLAAVKKANAA) and PAc (346–364) (AALTAENTAIKQRNENAKA) were derived from the sequence of the PAc gene from *S. mutans* MT8148, as reported by (Okahashi *et al.*, 1989). The peptide was synthesized using a stepwise solid-phase procedure at Asahi Techno Glass Co. Inc. (Tokyo, Japan). Synthesized peptide samples were subsequently purified by reversed-phase HPLC on a TSK-GEL column (1 × 30 cm) (TOSO, Tokyo, Japan) with a 10–45% acetonitrile gradient in 0.1% trifluoroacetic acid (TFA) and developed

over 50 min at a flow rate of 5 mL min^{-1}. Purity was determined to be greater than 95% in each tube by HPLC analysis. To confirm the amino acid sequences of the synthetic peptides, several samples were randomly selected, and then analysed using a System 7300 Amino Acid Analyzer (Beckman, NJ) and a Model 477A Protein Sequencer (Applied Biosystems, Foster City, CA). The peptide was used as a coating antigen in enzyme-linked immunosorbent assay (ELISA) examinations to determine the antibody titre in the saliva samples.

Human saliva collection

Whole saliva samples were collected on swabs after stimulating by biting paraffin gum for 5 min and placed in transport fluid (0.4% agar, 0.15% thioglycolate/phosphate-buffered saline), to determine the numbers of mS and tS, which was performed by Bio Medical Laboratory (BML, Tokyo, Japan). Other saliva samples was also collected after stimulation by biting paraffin gum and placed into ice-chilled sterile bottles over a period of 5 min, which were then clarified by centrifugation at 10 000 *g* for 10 min, filter-sterilized and used immediately for measuring the antibody levels.

ELISA

For enumeration of the IgA specific to *S. mutans*, 96-well microtitre H-plates (Sumitomo Bakelite, Tokyo, Japan) were coated overnight at 4 °C with 100 μL of PAc (361–386) and PAc (346–364) peptides (concentration 20 μg mL^{-1}) or skimmed milk (as a control) in coating buffer at pH 9.6, and then subjected to ELISA (Senpuku *et al.*, 1996b). The plates were washed with phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 (PBST) and blocked with 1% (w/v) skimmed milk in PBST for 1 h at 37 °C. Excess skimmed milk was removed by washing three times with PBST, and then a 100-μL aliquot of a 1 : 4 dilution of saliva was added to the wells and the mixtures were incubated for 1 h at 37 °C. The wells were then washed five times with PBST and further incubated for 1 h at 37 °C with 100 μL of alkaline phosphatase-conjugated goat antihuman immunoglobulin A (both heavy and light chains) antibodies (Zymed Laboratories, South San Francisco, CA). After five washes with PBST, bound antibodies were detected after the addition of 100 μL of para-nitrophenyl phosphate at 3 mg mL^{-1} as a substrate and incubation for 90 min at 37 °C. Absorbance at 405 nm was measured with a microplate reader (Multiskan Bichromatic Laboratory Japan, Tokyo, Japan). The experiments were performed independently three times, with similar results obtained in each.

Bacterial counts

Cotton swabs containing saliva samples from the elderly subjects were placed in transport fluid and taken to BML for analysis. Each sample was poured onto Mitis–Salivarius agar (Nippon Becton Dickinson Co. Ltd, Tokyo, Japan) or modified Mitis–Salivarius agar containing 0.2 U mL^{-1} of bacitracin (MMTSB) (Tsuha *et al.*, 2004) using an EDDY JET spiral plating system (IUL, S.A., Barcelona, Spain), and incubated at 37 °C under anaerobic conditions for 48 h, before counting tS and mS organisms. MMTSB is known to be extremely precise for the counting of mS colonies (Tsuha *et al.*, 2004). Following anaerobic inoculation for 48 h at 37 °C, numbers CFU were counted. Colonies of mS were identified by their characteristic appearance and the mS ratio was calculated as colony numbers of mS/colony numbers of tS × 100. All bacteria counting was performed by BML.

Blood parameters

Ten millilitres of blood was extracted from each of the elderly subjects, placed in a sterilized glass tube including heparin, and sent to Niigata Rinsyo Laboratory (Niigata, Japan) to determine the following blood parameters: leukocyte count, erythrocyte count, haemoglobin concentration, haematocrit count, platelet count, mean cellular volume (MCV), mean cellular haemoglobin (MCH), mean cellular haemoglobin concentration (MCHC) and total serum IgA. A percentage of the different types of leukocytes was generated based on the counts of eosinophils, basophils, lymphocytes, monocytes and neutrophils.

Group comparisons

The subjects were divided into two groups according to ELISA antibody titre, those with a PPA level ≤ 0.1 (PPA not detected group) and those with a PPA level >0.1 (PPA detected group). This grouping by antibody level has been used in a previous epidemiological study (Tsuha *et al.*, 2004). The Mann–Whitney *U*-test was used to compare mean periodontal status and blood parameters between the two groups. Fisher's correlation test was used for correlation among PPA level, oral bacterial status, basophils, eosinophils and periodontal status in females. Differences at $P = 0.05$ were considered to be significant. StatView for Macintosh (Version 10.0) was used for all statistical analyses.

Results

There were no significant differences for the various parameters (DMFT, tooth number, sound tooth number, saliva volume) between the two groups (data not shown). Table 1 shows the periodontal status of the two groups based on antibody level, as determined by ELISA. The average rAL6 result (8.7 ± 14.1) in the PPA detected group was significantly lower than that (14.7 ± 22.9) in the PPA not detected group. In addition, females in the PPA detected group had lower values for average BOP, AL, rAL6 and rPD (8.9 ± 11.1,

Table 1. Periodontal status of subjects in PPA detected and non-detected group

Periodontal status	PPA non-detected group ($n=40$)	PPA detected group ($n=218$)	P-value*
rCA	1.6 ± 4.6	1.1 ± 3.3	0.864
BOP	11.4 ± 13.1	8.9 ± 11.5	0.348
AL	3.7 ± 1.3	3.4 ± 1.0	0.220
rAL4	44.1 ± 32.6	37.1 ± 28.1	0.236
rAL6	14.7 ± 22.9	8.7 ± 14.1	0.035*
RPD	2.2 ± 0.1	2.2 ± 0.5	0.989
rPD4	11.6 ± 13.9	10.4 ± 11.9	0.882
rPD6	2.4 ± 4.4	2.0 ± 4.5	0.555

*Significant difference demonstrated by Mann–Whitney U test

3.4 ± 1.0, 9.0 ± 13.8 and 2.2 ± 0.5, respectively, $n=99$) as compared with those in the nondetected group (16.8 ± 17.2, 4.1 ± 1.7, 22.1 ± 32.6 and 2.5 ± 0.7, respectively, $n=13$) but these differences were not significant. Antibody specificity to the PAc (361–386) peptide was also confirmed by comparisons between PPA and the anti-PAc (346–364) peptide antibody (positive control) (Senpuku *et al.*, 1996b), and the antiskimmed milk antibody (negative control). The PAc (346–364) peptide has an antigenic epitope that recognizes human antibodies (Senpuku *et al.*, 1996b), but no correlation was found between this and numbers of mS, tS and LB organisms in saliva or the various periodontal status parameters (data not shown). There were no significant differences between males in the two groups for any of the periodontal parameters. For female subjects, to clarify the systemic association of PPA with periodontal status, various blood status parameters, such as leukocytes, erythrocytes, haemoglobin and haematocrit, were tested and compared between the two groups. The PPA detected group had significant lower values for eosinophils (2.5 ± 1.8%) and basophils (0.5 ± 1.3%) than the PPA not detected group (4.0 ± 2.0% and 0.8 ± 0.3%, respectively) (Table 2). However, there were no significant differences between the two groups when only males were analysed. For females, PPA showed a positive correlation with LB and tS counts and a negative correlation with proportion of basophils ($P=0.0015$, 0.0433 and 0.0340, respectively) (Table 3). The number of tS organisms also showed a positive correlation with LB and mS numbers ($P=0.0004$ and <0.0001, respectively), while the number of LB organisms showed the largest correlation with number of mS in all comparisons ($P=0.0170$). The percentage of eosinophils was positively correlated with that of basophils, as well as AL, BOP and rPL6 ($P=0.029$, 0.0439, 0.0475 and 0.0168, respectively). However, there were no significant differences between PPA level and total serum IgA level (data not shown). Thus, it was clear that PPA level in saliva responded to the progression of periodontal disease, as well as to the proportions of eosinophils and basophils in female subjects.

Table 2. Blood status between PPA detected and non-detected female subjects

	PPA non detected group ($n=13$)	PPA detected group (104)	P-value
Leukocyte ($\times 10^3\,\mu L^{-1}$)	5.9 ± 1.3	5.8 ± 1.4	0.118
Erythrocyte ($\times 10^6\,\mu L^{-1}$)	4.2 ± 0.3	4.0 ± 0.4	0.187
Hemoglobin ($g\,dL^{-1}$)	39.2 ± 3.2	12.3 ± 1.4	0.362
Hematocrit (%)	12.6 ± 1.1	38.0 ± 3.9	0.302
Platelet ($\times 10^4\,\mu L^{-1}$)	21.1 ± 5.7	21.1 ± 5.4	0.948
MCV (fl)	93.8 ± 3.7	94.4 ± 4.0	0.475
MCM (pg)	30.2 ± 1.4	30.4 ± 2.6	0.390
MCHC (%)	32.2 ± 0.7	32.4 ± 2.6	0.948
Eosinophil (%)	4.0 ± 2.0	2.5 ± 1.8	0.005*
Basophil (%)	0.8 ± 0.3	0.5 ± 0.3	0.003*
Lymphocyte (%)	33.7 ± 6.9	36.9 ± 6.9	0.192
Monocyte (%)	5.6 ± 1.0	5.6 ± 1.4	0.882
Neutrophil (%)	56.0 ± 8.2	54.5 ± 6.6	0.578

*Significant difference demonstrated by Mann–Whitney U test

Table 3. Correlation among PPA level, oral bacteria status, basophil, eosinophil and periodontal status in female

	Correlation	P-value
PPA vs. LB	0.297	0.0015*
PPA vs. tS	0.193	0.0433*
PPA vs. mS	0.044	0.6484
tS vs. LB	0.328	0.0004*
tS vs. mS	0.423	< 0.0001*
LB vs. mS	0.227	0.0170*
PPA vs. Eosinophil	−0.119	0.2160
PPA vs. Basophil	−0.195	0.0340*
Eosinophil vs. Basophil	0.272	0.0029*
AL vs. Eosinophil	0.192	0.0439*
BOP vs. Eosinophil	0.189	0.0475*
rAL6 vs. Eosinophil	0.227	0.0168*

*Significant differences demonstrated by Correlation Fisher' Test

Discussion

The present results suggest that the presence of PPA indicates immunological activities that induce the production of human IgA antibodies to tS, but not those to mS, in elderly subjects. A positive correlation between antibody levels in saliva and previous infection and colonization with tS containing *S. mutans* and *S. sobrinus* in the oral cavity is suggested. Furthermore, a high concentration of PPA has been speculated to play a role as a negative contributor in proportion to the numbers of mS organisms in the oral cavity (Tsuha *et al.*, 2004). However, in the present study, the mS ratio was higher in the PPA detected group than in the PPA not detected group for all subjects, as well as for female and male subjects separately (1.5 ± 3.7 vs. 1.0 ± 1.8, 1.7 ± 3.9 vs. 1.3 ± 1.9 and, 1.4 ± 3.4 vs. 0.8 ± 1.8, respectively),

although the differences between the two groups were not significant.

Amino acid residues 365–377 [PAc (365–377) peptide] in the A-region is an antigenic epitope for the induction of antibodies that inhibits the interactions of *S. mutans* PAc with human salivary components (Senpuku *et al.*, 1995, 2001). Furthermore, the common epitope (YEA-L-QY) between the surface protein antigen (PAg) of *S. sobrinus* (Okahashi *et al.*, 1993) and its PAc, as well as its core B-cell epitope (–Y–––L––Y–) are essential sequences in the antigenic epitopes of the surface proteins of oral streptococci that are specifically recognized by the antibody (Senpuku *et al.*, 1997). The antibody to the core epitope in PPA may serve as an indicator of infection by tS as well as mS colonization. Because PPA plays roles as a positive indicator and negative effector of mS infection, the noncorrelation between PPA level and mS count in saliva seen in our study might have been caused by the combination of positive and negative effects. In addition, the correlations between numbers of LB organisms and PPA, tS and mS indicate that LB may be incorporated with tS biofilm formation.

Periodontitis often induces bone absorption and gingival recession through gingival inflammation. In addition, gingival recession changes the oral condition, which may cause ecological changes, resulting in microbiological changes such as the development of supragingival plaque-containing streptococcal bacteria (Quirynen *et al.*, 1999; Reiker *et al.*, 1999). Marsh found that the optimal growth conditions for streptococci were different from those for other periodontal pathogens (Marsh, 2003), while other studies have shown that the growth of periodontal pathogens is not correlated with or inhibited by *S. mutans*, and also that the number of streptococci was important for growth conditions for periodontal pathogens (Drake *et al.*, 1993; Grenier, 1996). Therefore, an increase in tS number may produce PPA and inhibit the growth of microorganisms that are associated with the progression of periodontal diseases.

In the present study, the PPA detected group showed a significantly lower number of sites with rAL6 than the PPA not detected group, while the PPA detected group also showed a lower rate of AL in female subjects. Several studies have reported a relationship between CA and AL (Albandar *et al.*, 1996; Timmerman *et al.*, 2000; Neely *et al.*, 2001; Cobb, 2002); however, our PPA not detected group showed a significant progression of AL, but not of CA deposition. Therefore, there may be contrasting variables involved in the relationships between the antibody and AL increases and reduction with ageing. We performed a comparison between PPA and periodontal status in a small number of elderly subjects over a 4-year period (initial study in 2001; average age 73 years; 60 males, 27 females). Two years after obtaining baseline data, 62 of the subjects from the original cohort participated in follow-up examinations (2003 study; average age 75 years; 45 males, 17 females) and 1 year later 69 subjects from the original cohort (2004 study; average age 76 years; 46 males, 23 females) also participated, with periodontal status continuously observed in the PPA detected and PPA not detected groups. We found that AL was significantly lower in the PPA detected group in all subjects at every examination (data not shown), indicating a consistent association of AL with induction of PPA.

In the present study, eosinophils were correlated positively with periodontal status during the progression of periodontitis, while basophils were also correlated positively with eosinophil and negatively with PPA levels. Eosinophils and basophils are known to be associated with most inflammatory and infectious disorders associated with allergic manifestations. The eosinophil fraction in gingival crevicular fluid (GCF) from periodontitis patients has been reported to range from 6 to 10%, which is much higher than that in circulating blood (Sugita *et al.*, 1993), while GCF specifically yielded a higher rate of activated eosinophils in another study (Suzuki *et al.*, 1995). A striking common feature of many autoimmune and inflammatory diseases in humans is that females are more susceptible to specific immunological disorders than males (Ansan Ahmed *et al.*, 1985). Our results support those data, and also suggest that eosinophils and basophils play important roles in host immune and defensive systems in response to periodontitis in elderly females.

The present findings suggest that the anti-PAc (361–386) peptide antibody is responsible for physical status in periodontal tissues, as well as systemic condition with regard to eosinophil and basophil proportions among leukocytes during the development of periodontal disease. They also imply that the induction of PPA may be indirectly correlated with some of the factors that inhibit periodontal pathogens. We conclude that this antibody is useful as a predictor of periodontal diseases in elderly patients.

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特集 歯科および口腔内の感染症の診断と治療

2. 歯科ユニットのバイオフィルム

泉福 英信*

歯科医療を行うにあたって，その安全性の確保は最も重要な課題であるが，院内感染のリスクは未だ減少しておらず，その監視体制の整備が望まれている。院内の環境や医用材料・医療機器に形成されるバイオフィルムは消毒薬に抵抗性を示し，そのことが院内感染の要因になっている可能性が高い。近年諸外国を含め日本でも，歯科用ユニットの給水管内（ハイスピードハンドピース，スリーウエイシリンジおよび超音波スケーラーへ水を送る細い内径のプラスチックチューブ）にバイオフィルムが形成され，そこから出てくる水に含まれる微生物数は，水道水よりもはるかに多いことが院内感染のリスクになると指摘されるようになってきた。患者，術者等が歯科診療中に感染しないようにするためには，院内感染対策の基本であるユニバーサルプレコーション（歯科医療ではスタンダードプレコーションと同義に用いられる）のもとに医療を行う必要がある。そのためには，歯科ユニットから出てくる水に対してもより安全な方法を選択するために，微生物数を減少させる手段をとる必要がある。過酸化水素水，クロルヘキシジン，過酢酸等の化学物質を併用して歯科ユニット内バイオフィルムを限りなく減少させる手段が今後重要である。

Key Words：バイオフィルム／院内感染対策／歯科ユニット／レジオネラ／化学物質

I はじめに

院内におけるバイオフィルム形成菌は，外因的ないし内因的要因により供給される。外因的要因として，緑膿菌，レジオネラ，非結核性非定型抗酸菌などのヒトに対して病原性のある菌が，歯科用ユニットの給水管内（ハイスピードハンドピース，スリーウエイシリンジおよび超音波スケーラーへ水を送る細い内径のプラスチックチューブ）に存在していることは明らかになっており[1]，歯科医療従事者が慢性的にレジオネラに暴露されている例が報告されている[2]。また，癌治療中の免疫低下した患者が汚染歯科用冷却水の暴露により緑膿菌感染した例も報告されている[3]。一方，内因的要因として，鼻腔や口腔には500種類以上の微生物が潜んでおり，加齢，全身状態の悪化，口腔清掃習慣の不良，義歯の着用，薬使用により，正常細菌叢が崩れ，MRSA（メチシリン耐性黄色ブドウ球菌），セラチア菌，緑膿菌，肺炎桿菌，真菌などの日和見菌による感染が考えられている。重度の歯周病患者や高齢者の場合，これら日和見菌が検出される割合も高く，また重度の歯周病では出血し，その中に含まれる病原性ウイルス（HIV〔ヒト免疫不全ウイルス〕，HCV〔C型肝炎ウイルス〕，HBV〔B型肝炎ウイルス〕など）も口腔外へ噴出した際に感染リスクが増大してくる。一方，口腔バイオフィルム細菌などが口腔外へ飛散すれば，様々な器具や給水管などに付着してバイオフィルを形成していく。本稿では，歯科ユニットの配水管のバイオフィルム汚染について海外の研

Biofilm in dental unit

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究を参考にその対策を解説し，厚生労働科学研究班において行った歯科医院における感染対策の調査の一部も紹介する。

Ⅱ　配水管内のバイオフィルム

歯科用医療器具に形成されたバイオフィルムは，洗浄，消毒，滅菌等の方法によりその感染力を減弱させられるが，給水管の中にできたバイオフィルムを除去あるいは減少させるのは容易ではない。新しく設置された歯科ユニットの給水系には，設置後5日以内に1mL当たり20万以下のCFU（colony forming unit）の微生物に達すると言われている[4]。1993年に，CDC（米国疫病予防管理センター）は，配水の微生物を減らすため，診療開始時に歯科配水管から一気に配水するように勧告した[5]。しかし，一時的に菌数は減るもののこの処置は給水管のバイオフィルムに影響を与えず，またすぐにもとに戻ることが指摘された[6]。1996年にアメリカ歯科医師会は歯科用水に関して，給水管から送られる水がフィルターなしで細菌が200CFU/mL以下になるように推奨した。ヨーロッパにおけるガイドラインでは，100CFU/mLを推奨している。しかし約90%の歯科ユニットにてこの基準値を満たしていないことも明らかとなった[7]。日本において，厚生労働省の研究班（歯科医療における院内感染対策，主任研究者筆者）では，某県歯科医師会に所属する3,912名にアンケート調査を行い，有効回答のあった742名（19%）のアンケート結果を分析すると，66%の歯科医師が歯科ユニットから出てくる水が水道水よりも微生物が多く含まれていることを認識していた[8]。しかし，日本において特に歯科ユニットからの配水の微生物推奨基準値は定められておらず，厚生労働科学研究班の中でその対策が練られようとしている。また論文も数報報告されてきており[9,10]，今後その認識が高まっていくものと考えられる。

Ⅲ　バイオフィルム微生物

配管内バイオフィルム微生物は，大きく分けて細菌，真菌，原虫などであることがわかっている[11]。これらの微生物の供給源は，水道水，歯科ユニット周囲の環境，ヒト口腔からの微生物，唾液や血液などが考えられている（図1）。具体的な微生物は，その大半が水中従属栄養細菌[4,12,13]で，これらは正常な免疫応答をする能力のあるヒトには，病原性となる可能性はほとんどない。さらに口腔常在菌[11]，ヒト病原菌（緑膿菌[4,11,13]，レジオネラ菌類[11,14]および非結核性抗酸菌類[11]など）が分離されている。特にレジオネラは，エアロゾルに運ばれ感染を促していくのでタービンを使用する時は注意を払う必要がある。一方，病原性微生物は検出された数例が示されているだけでほとんど検出されない場合も多く，また日本で検出された報告がない。しかし，全身疾患を有するような易感染者に対して感染リスクが高くなることやスタンダードプレコーションの概念を考えれば，より安全を期するために歯科ユニットからの配水に微生物の多いことに対して認識を高め，定期的な検査や微生物を減少させるための処置を考えていく必要がある。

Ⅳ　水質改善の方法

歯科ユニットの配水から微生物を減らすために，診療開始前に一気に排水をする方法が1993年にCDCにより勧告された[5]。しかし，この方法ではアメリカ歯科医師会が推奨する200CFU/mL以下に到達するユニットは1%以下に過ぎず[15]，給水管のバイオフィルムに影響を与えず，水質を信頼できるほど改善させられないことが明らかとなった。一方，歯科診療前に5分間の過酢酸を用いた化学的な処理により，200CFU/mL以上を示す歯科ユニットは約9%にまで減少し，十分な微生物の制御ができることが証明された[16]。化学物質により持続的あるいは間欠的に水を処理するための内蔵型給水装置が考案された。そこに使用される化学物質として，塩素[17]，グルコン酸クロルヘキシジン[18]，過酸化水素水[19]，イソジン[12]，市販のマウスリンス[20]が使用された。しかし，持続的に処理するため，器具や歯科材料の腐食や損傷および術者への障害を考慮に入れると低濃度でないと使用できず，その結果効果が弱いため診療中に吸引された微生物を瞬時に殺菌することができない。診療後や診療前のみに高濃度の化

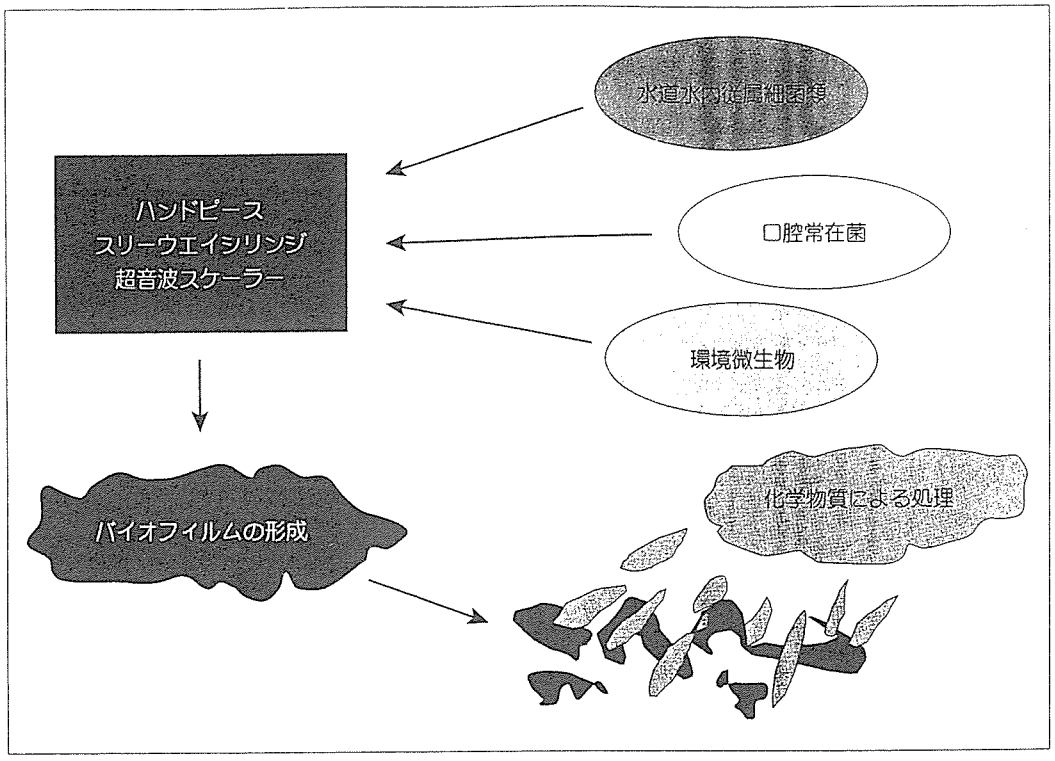

図1　歯科ユニットのバイオフィルム形成とその除去

微生物の供給源は，水道水，歯科ユニット周囲の環境，ヒト口腔からの微生物，唾液や血液などが考えられている。

学物質で処理する方法も効果的であるが，診療中の微生物汚染は防ぐことができない。また近年では，優れた化学物質として tetraacetylethylenediamine（TAED）の使用が検討された。これは，過酢酸の前駆物質で過酢酸のような副作用を持っておらず，中性に近い pH で，炎症を引き起こしたり悪臭もなく安価な物質として注目されている。この物質の使用により，歯科診療中の微生物汚染や配水管内におけるバイオフィルム形成を制御できることが明らかとなった（図1）[16]。

V　配水管処理に使用される化学物質のまとめ

2003 年に JT Walker らがまとめた世界で購入できる歯科ユニット配水管に使用可能な化学物質のリストを挙げる（表1）[21]。

彼らは，実験的にこれらの化学物質が配水管上のバイオフィルムに効果的作用するか 14 日間の検討を行った。Combizyme と Ozon は，完全にバイオフィルムを除去できないが 45%と 57%程度の除去を示した。クロルヘキシジン，Bio2000 や Tegodor は，31，53，33%のバイオフィルム除去を示した。Dialox®，Betadine®，Alpron，Sporklenz，Sterilox，Sanosil®，Oxigenal，Grotanat® Bohrerbad は 95%以上のバイオフィルム除去を示した。これらは，実験的なバイオフィルムに対しては高い効果を示したが，実際の歯科ユニットへ作用させたときにどの程度効果が出てくるか今後の検討が必要としている。

VI　厚生労働科学研究班での解析

2004 年度から，歯科医療における院内感染対策の厚生労働科学研究班がスタートした。その中で某首都圏の歯科医師会所属 3,912 人に対して院内感染対策のアンケート調査を行い，有効回答のあった 742 名（19%）のアンケート結果を分析すると，66%の歯科医師が歯科ユニットから出てくる水が水道水よりも微生物が多く含まれていることを認識していた[8]。またそのことを認識していた歯科医師は，院内感染対策の基本であるユニバーサルプレコーションの理解と有意に相関が認められ，さらに院内感染対策の卒後研修を受けたこと

表1　歯科ユニット配水管の微生物減少に使用できる化学物質のリスト

化学物質（商品名）
1-2%次亜塩素酸ナトリウム，70%クエン酸（Alpron）
12%エタノール，0.12%クロルヘキシジン（Bio2000），0.2%クロルヘキシジン
1.25%プロテアーゼ（Combizyme），1%過酸化水素水（Dentasept）
過酸化水素水，過酢酸，酢酸（Dialox®），0.4%過酸化水素水（Oxigenal）
水酸化カルシウム，プロパノール，エチルヘキサノール（Grotanat® Bohrerbad）
200 mg/h オゾン（Ozon），10%ポビドンヨード液（Betadine®）
5%過酸化水素水，銀（Sanosil®），0.5%亜塩素酸塩（Sodium hypochlorite）
過酸化水素水，過酢酸，酢酸（Sporklenz），2.5%超酸性水（Sterilox）
1%塩化ベンザルコニウム，ホルムアルデヒド，グルタールアルデヒド（Tegodor）

やスタッフへの院内感染対策教育を行っていることにも有意な相関が認められた。よって，歯科ユニット配水のバイオフィルムによる微生物汚染の認識は，院内感染対策の指標となりうると考えられた。

Ⅶ　おわりに

歯科ユニットのバイオフィルム汚染の問題に止まらず，歯科医療における院内感染対策の啓蒙・啓発運動が重要と考えている。その一つとして，バイオフィルム除去方法の確立は急務となっている。より安全で安価な化学物質の開発を含めた歯科ユニットの水質改善の方策ができれば，より容易に院内感染対策を行動科学的導入できるようになると考える。

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