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Effect of non-surgical periodontal treatment on short chain fatty acid levels in gingival crevicular fluid of patients with generalized aggressive periodontitis

R. Lu[1], H. Meng[1], X. Gao[2], L. Xu[1], X. Feng[1]

[1]Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, China and [2]Department of Cariology and Endodontology, Peking University School and Hospital of Stomatology, Beijing, China

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Background and Objective: Short chain fatty acids (SCFAs) play important roles in periodontal diseases. However, the concentrations of SCFAs in gingival crevicular fluid of patients with aggressive periodontitis are not known. The aim of this intervention study was to investigate the influences of non-surgical periodontal therapy on levels of SCFAs in the gingival crevicular fluid of patients with generalized aggressive periodontitis (G-AgP), and analyze the concentrations of SCFAs in sites with or without the detected putative periodontal pathogens.

Material and Methods: Eighty gingival crevicular fluid samples (four per subject) were collected on filter paper strips from patients with G-AgP (n = 20; mean age 24.5 years), before and at 2 wk, 2, 4 and 6 mo after non-surgical periodontal treatment. Eighty gingival crevicular fluid samples (four per subject) were collected from periodontally healthy controls (n = 20; mean age 26.2 years). Concentrations of formic acid, succinic acid, acetic acid, lactic acid, propionic acid, butyric acid and isovaleric acid from the supernatant of gingival crevicular fluid samples were measured by high performance capillary electrophoresis. *Porphyromonas gingivalis*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Fusobacterium nucleatum* from the precipitate of the same pretreatment samples of gingival crevicular fluids were analyzed by polymerase chain reaction amplification.

Results: The clinical parameters of patients with G-AgP during the 6 mo after non-surgical periodontal treatment were improved remarkably. The formic acid concentration increased significantly after treatment; the level of formic acid was lower in the *P. gingivalis*-, *T. denticola*-, *P. intermedia*- or *F. nucleatum*-positive sites compared with the negative sites. The concentrations of acetic acid, propionic acid and butyric acid reduced significantly after treatment and reached the lowest level at 2 wk post-treatment, although showed a tendency to increase after 2 mo post-treatment, and the three SCFA levels were significantly higher in *P. gingivalis*-, *T. denticola*-, *P. intermedia*- or *F. nucleatum*-positive sites compared with those in the negative sites.

Huanxin Meng DDS, PhD, Department of Periodontology, Peking University School and Hospital of Stomatology, 22 Zhongguancun Nandajie, Haidian District, 100081 Beijing, China
Tel: +0086 10 82195368
Fax: +0086 10 62173402
e-mail: kqhxmeng@bjmu.edu.cn

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Conclusion: Non-surgical periodontal treatment resulted in a significant decrease of acetic acid, propionic acid, butyric acid levels and increase of formic acid level in gingival crevicular fluids in patients with G-AgP, accompanied by improvement in clinical parameters. A marked lower level of formic acid, as well as higher levels of acetic acid, propionic acid and butyric acid in gingival crevicular fluid of patients with G-AgP was consistent with periodontal pathogen infection.

Generalized aggressive periodontitis (G-AgP) is an inflammatory disease that has its onset primarily during early adult years, and is characterized by rapid attachment loss and bone destruction (1). Although G-AgP is relatively rare in the general population, there is considerable interest in studies aimed at understanding the etiology and pathogenesis of this disorder. Extensive research has been carried out on identifying useful biomarkers in the gingival crevicular fluid to manifest the progression of G-AgP (2–4); however, less is known about short chain fatty acids (SCFAs) in gingival crevicular fluids in patients with G-AgP.

SCFAs, such as formic acid, succinic acid, acetic acid, lactic acid, propionic acid, butyric acid and isovaleric acid are normally found in human intestines (5,6) and play significant roles in diabetes (7,8), colon cancers (9) and obesity-related inflammation (10). SCFAs can also be fermentation products of anaerobic bacteria and found in sites associated with infection, such as periodontal disease (11–13). It was confirmed by *in vitro* studies that putative periodontal pathogens, such as *Porphyromonas gingivalis*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Fusobacterium nucleatum* can produce SCFAs (14,15). Other oral bacteria, such as *Streptococcus mutans* (16) and *Prevotella histicola* sp. nov. (17) can also produce SCFAs. Millimolar levels of propionic acid and butyric acid have been found in gingival crevicular fluid from patients with chronic periodontitis (12,13,15), and this may disable the tight attachment among epithelial cells, resulting in bacterial penetration and periodontal tissue destruction (18).

The biological effects of SCFAs have been an area of extensive investigation. It is reported that butyric acid and propionic acid could modulate neutrophil function (19), and diminish tumor necrosis factor-alpha production by lipopolysaccharide-stimulated neutrophils (20). SCFAs can damage immunoregulatory cells, e.g., butyric acid produced by *P. gingivalis* and *F. nucleatum* can induce apoptosis in human monocyte and macrophage cell lines (21), impair cell growth and cell cycle progression in gingival fibroblasts (22), and thus are involved in the pathogenesis of periodontal diseases. Butyric acid is responsible for HIV-1 reactivation via chromatin modification by *P. gingivalis* (23). SCFAs may also contribute to biological interactions among oral bacteria (16).

The effects of SCFAs on cell growth may be manifested as inhibition or stimulation (18), butyric acid has a biphasic stimulation of monocyte interleukin-1 beta production (24), which depends on the concentration of SCFAs. Our group has previously analyzed the effect of periodontal therapy on the levels of SCFAs in patients with chronic periodontitis, which demonstrated the SCFA levels change in gingival crevicular fluids after treatment that are consistent with the reduction of periodontal inflammation (13). However, the concentrations of SCFAs in gingival crevicular fluids in patients with G-AgP, as well as their relationships with putative periodontal pathogens, are not well understood. Therefore, this case–control, intervention study was performed to investigate, for the first time, the change of SCFA levels in the periodontal pockets of patients with G-AgP before and after non-surgical periodontal treatment. The results of this study are expected to provide useful information on the effects of SCFAs in G-AgP.

Material and methods

Study population

Twenty patients with G-AgP were recruited consecutively from the Department of Periodontology, Peking University School and Hospital of Stomatology from February 2010 to May 2011. All subjects were never-smokers and in good health, as confirmed by physical examination and a comprehensive blood examination. The diagnostic criteria for G-AgP were based on the classification proposed at the International Workshop for the Classification of Periodontal Diseases and Conditions in 1999 (1). The details were that (i) patients were < 35 years old, and presented at least 20 teeth in the mouth, and (ii) they had at least eight teeth with a probing depth over 6 mm, radiographic evidence of alveolar bone loss and at least three of the teeth were not the first molars or incisors (4).

The inclusion criteria of healthy controls were ≥ 18, and < 35 years old, good oral hygiene, no clinical evidence of periodontitis (probing depth ≤ 3 mm; whole mouth bleeding on probing sites $< 10\%$; no attachment loss caused by periodontal destruction).

Exclusion criteria for the two groups were: (i) pregnancy or lactation period; (ii) intake of antibiotics or anti-inflammatory drugs in the previous 3 mo; (iii) systemic diseases; (iv) history of periodontal treatment in the preceding 6 mo; (v) smoker; and (vi) history of orthodontic

treatment or significant occlusal disharmony. The study protocol was approved by the Ethics Committee of Peking University Health Science Center. Written informed consent was obtained from each subject in accordance with the declaration of Helsinki.

Sample size calculation

The sample size calculation was based on data from the study of Qiqiang *et al*. (13), using the software NCSS&PASS by "repeated measures of means". Considering that the butyric acid concentration was 3.0 mM before periodontal treatment, 1.5 mM at 2 wk post-treatment and 2.0 mM at 2, 4 and 6 mo post-treatment respectively, the repeated measures were set to be five times. Then, at least 15 subjects would be necessary to provide 85% power with an alpha of 0.05. Based on the mean dropout rate of 15% from our previous studies, 20 subjects were included in each group.

Study outline and clinical examination

After an initial screening visit for recruitment, all individuals were reappointed for collection of gingival crevicular fluid samples 1 wk later, and this was defined as the baseline. Then, patients with G-AgP received full mouth supragingival scaling, oral hygiene instruction, quadrant-based scaling and root planing under local anesthesia within 1 mo, lasting approximately 1 h each time. Patients were recalled at 2 wk, 2, 4 and 6 mo post-therapy, and gingival crevicular fluid samples were collected from the same sites as those for baseline. Individualized oral hygiene instruction, clinical measurements and supragingival scaling were also performed at 2, 4 and 6 mo post-therapy.

Clinical parameters were assessed by a calibrated examiner. The probing depth and attachment level (AL) were recorded at six sites around each tooth, except the third molars, to the nearest millimeter, using a UNC-15 probe tip (Hu-Friedy, Chicago, IL, USA), bleeding index (BI) (25) was recorded at 30 s after probing. The plaque index (26) was recorded as the highest value at buccal and lingual side per teeth. Ten non-study subjects with moderate to severe chronic periodontitis were recruited as subjects for the calibration exercise before the study. The single designated examiner measured the full mouth probing depth and AL, except the third molars. Within 3 d, the examiner repeated the measurements. The results showed 99.7% reproducibility within $\pm$ 1 mm in probing depth measurements and 99.0% reproducibility within $\pm$ 2 mm in AL measurements. All the clinical parameters, before and after non-surgical periodontal treatment were measured by the same examiner.

Gingival crevicular fluid sampling

At baseline and each follow-up time point, gingival crevicular fluid samples were obtained by a single calibrated investigator from one mesiobuccal site in each quadrant, which is one in the upper incisor and three in first molars in the other three quadrants (G-AgP: probing depth $\geq$ 5 mm; control group: probing depth $\leq$ 3 mm). The tooth selected was not an abutment tooth, with no overhang fillings or food impaction. If the first molar was not available, a second molar or a premolar in the same quadrant was selected. The sampling of gingival crevicular fluids was between 08.00 and 10.00 h from subjects who had fasted overnight. After the supragingival deposits were carefully removed by a curette, the sites for sampling were isolated with cotton rolls to avoid saliva pollution and gently air-dried. Then, a filter paper strip (10 $\times$ 2 mm, 3MM, Whatman Ltd., Maidstone, Kent, UK) was placed in the pocket until mild resistance was felt, and then left in place for 30 s. Strips that were visibly contaminated with blood were discarded. The filter strips were placed in a sterile Eppendorf tube that had been weighed before sampling, and again immediately after sampling in an airtight room, and sealed with parafilm to avoid evaporation. The samples were stored at -80°C until assayed. Both weighing operations were carried out on an analytical balance with a sensitivity of 0.01 mg (AE 240s; Mettler, Zurich, Switzerland). The difference between the weights was used to calculate the volume of gingival crevicular fluids (4,13). Gingival crevicular fluid samples were collected in the same way from the subjects in the healthy control group.

Analysis of short chain fatty acids in gingival crevicular fluids

On the day of the assay, deionized water 100 times the sample volume was added to the tubes containing the sample strip and thawed at room temperature, then vibrated at 600 rpm at 20°C on an Eppendorf Thermomixer Vomfort for 20 min and centrifuged for 15 min at 10,000 *g*. The supernatant was separated and used for the detection of SCFAs, including formic acid, succinic acid, acetic acid, lactic acid, propionic acid, butyric acid and isovaleric acid. The SCFA analysis was done by high performance capillary electrophoresis (HPCE) (Beckman, P/ACE system 5000; Beckman Coulter, Fullerton, CA, USA), as reported by Qiqiang *et al*. (13). Briefly, the separation was carried out at a constant temperature of 20 $\pm$ 0.1°C, in a silica capillary tube 57 cm in length and 75 μm in diameter, under a voltage of 20 kV with reversed polarity. The gingival crevicular fluid supernatant in an electrolyte (containing 10 mM phthalic acid and 0.5 mM tetradecyl trimethyl ammonium bromide and adjusted to pH 5.7 with 0.1 mM lithium hydroxide) was injected at a pressure of 0.5 psi at a rate of 6 nL/s for 10 s. The difference in ultraviolet absorption (254 nm) of the ions of interest was measured as a function of migration time. Standard curves were obtained from the analysis of analytically pure formic acid, succinic acid, acetic acid, lactic acid, propionic acid, butyric acid and isovaleric acid (Beijing Chemical Co., Beijing, China) mixtures in 2 mM *o*-phthalic acid buffer (pH 5.7, adjusted with lithium hydroxide). The linearity of the calibration curves for the seven different SCFAs was excellent (R^2 = 0.99) within the concentration range of the standard.

Stability analysis of SCFAs by HPCE was done before the analysis, and the precision, expressed in terms of the relative standard deviation, was from 1.6% to 7.7% for the seven kinds of SCFAs. The accuracy of the method was evaluated from the recovery analysis and ranged from 86% to 122%. Concentration data were given in mM. The detection limit of the HPCE was 10^{-13} mM.

Analysis of periodontal microorganisms

The precipitate of the pretreatment gingival crevicular fluid sample (including subgingival plaque) from patients with G-AgP was washed with 200 μL TE buffer (10 mM Tris–HCl, pH 7.6, 1 mM EDTA). Genomic DNA of bacteria was isolated with a commercial bacteria DNA mini kit (W6511; Watson Biotechnologies, Shanghai, China). Bacterial 16S ribosomal DNA of *P. gingivalis*, *T. denticola*, *A. actinomycetemcomitans*, *F. nucleatum* and *P. intermedia* was amplified by polymerase chain reaction (PCR), using primers and the PCR protocol described by Ashimoto *et al.* (27). The primer was synthesized on a Beckman DNA SM automated DNA synthesizer (SBS Genetechnology, Beijing, China). Twenty-five microliters of PCR reaction mixture was performed in a GeneAmp PCR system 2700 (ABI, Foster City, CA, USA). PCR products were analyzed by 1.5% agarose gel electrophoresis.

Statistical analysis of data

Clinical data were obtained in healthy control subjects at baseline and from patients with G-AgP from baseline to 6 mo after periodontal therapy. Mean values of clinical parameters were calculated for each individual and averaged across subjects in both groups. The mean percentage of sites with probing depth ≥ 7 mm and BI > 2 were also computed at baseline. The non-normally distributed data of SCFA concentrations were described as "median (lower–upper quartile)". Comparisons of the clinical parameters and the SCFA levels before and after treatment were analyzed using the Kruskal–Wallis test. Group comparisons were performed using the Mann–Whitney *U*-test. A software program (SPSS 11.5 for Windows; SPSS Inc., Chicago, IL, USA) was used for all calculations.

Results

Effect of non-surgical periodontal treatment on clinical parameters

The general status and clinical parameters of patients with G-AgP and healthy control groups were shown in Table 1, no significant differences were observed between the two groups for age, gender and the number of teeth in the mouth. The mean plaque index, probing depth, clinical attachment level and percentage of sites with BI > 2 or probing depth ≥ 7 mm at baseline were statistically significantly higher in patients with G-AgP compared with the healthy control subjects; all reached $p < 0.05$.

The effect of non-surgical periodontal treatment on clinical parameters in patients with G-AgP was presented in Table 2. Non-surgical periodontal treatment resulted in a significant improvement in the full mouth clinical parameters of patients with G-AgP, as indicated by the pronounced reduction of the plaque index (from 2.4 ± 0.4 to 1.2 ± 0.3), the percentage of BI > 2 sites (from 92.3 ± 16.2% to 11.9 ± 10.6%), probing depth (from 5.0 ± 0.9 mm to 3.0 ± 0.3 mm) and AL (from 4.1 ± 0.8 mm to 3.1 ± 0.3 mm) from baseline to the 6 mo post-treatment point ($p < 0.05$). Two patients with G-AgP were unavailable for the 4 mo post-therapy re-evaluation, and were not analyzed. All the clinical parameters of the healthy controls were significantly lower than those of patients with G-AgP at baseline ($p < 0.05$).

Effect of non-surgical periodontal treatment on short chain fatty acids levels in gingival crevicular fluid of patients with generalized aggressive periodontitis

The concentration of formic acid in gingival crevicular fluid of patients with G-AgP increased in a statistically significant manner at 2 wk, 2 and 6 mo post-therapy, compared with that in baseline ($p < 0.05$). The median of the formic acid concentration ranged from a low of 7.5 mM at baseline to a high of 10.2 mM at 2 mo post-treatment. The concentration of succinic acid, acetic acid, propionic acid, butyric acid and isovaleric acid remarkably decreased post-therapy, and the levels at 2 wk post-treatment were all statistically lower than those at baseline ($p < 0.05$). There was a significant increase of succinic acid, acetic acid and propionic acid levels at 4 mo post-therapy, and increase of isovaleric acid levels at 6 mo post-therapy compared with those at 2 wk post-therapy, although at a relatively

Table 1. Demographic data and clinical parameters (mean ± SD) of healthy controls and patients with G-AgP

	Control group (n = 20)	G-AgP group (n = 20)
Age (years)	26.2 ± 3.0 (range, 22–33)	24.5 ± 4.1 (range, 16–34)
Gender (male/female)	8/12	9/11
PI	1.1 ± 0.3	2.4 ± 0.4*
PD (mm)	1.9 ± 0.3	5.0 ± 0.9*
AL (mm)	< 1	4.1 ± 0.8*
BI > 2 (%)	3.4 ± 2.2	92.3 ± 16.2*
Sites with PD ≥ 7 mm (%)	0	30.5 ± 21.2*
Teeth (n)	28.0 ± 0.2	27.5 ± 1.1

AL, clinical attachment level; BI bleeding index; G-AgP, generalized aggressive periodontitis; PD, probing depth; PI, plaque index.
*$p < 0.05$, compared to the control group.

Table 2. Clinical parameters of the full mouth in patients with G-AgP at baseline, and at 2, 4 and 6 mo post-treatment

	Baseline (n = 20)	2 mo (n = 20)	4 mo (n = 18)	6 mo (n = 20)
PI	2.4 ± 0.4	1.7 ± 0.3*	1.5 ± 0.3*	1.2 ± 0.3*#
BI > 2 (%)	92.3 ± 16.2	27.1 ± 20.7*	22.0 ± 20.7*	11.9 ± 10.6*
PD (mm)	5.0 ± 0.9	3.3 ± 0.5*	3.2 ± 0.4*	3.0 ± 0.3*
AL (mm)	4.1 ± 0.8	3.4 ± 0.5*	3.2 ± 0.5*	3.1 ± 0.5*

AL, clinical attachment level; BI bleeding index; G-AgP, generalized aggressive periodontitis; PD, probing depth; PI, plaque index.
*$p < 0.05$, compared to baseline in patients with G-AgP.
#$p < 0.05$, compared to 2 mo post-treatment in patients with G-AgP.

low level compared with baseline. The median of succinic acid, acetic acid, propionic acid, butyric acid and isovaleric acid concentrations before and during the 6 mo following non-surgical periodontal treatment ranged from 1.4 mM to 0.4 mM, 26.0 mM to 7.6 mM, 8.8 mM to 3.7 mM, 2.5 mM to 0.0 mM, 2.0 mM to 0.5 mM, respectively. The lactic acid concentration ranged from 8.7 mM before treatment to 6.6 mM after treatment, the difference not being statistically significant, with $p > 0.05$. Details are shown in Table 3.

At 2 mo post-treatment, the probing depth in 36 of 80 sampling sites in patients with G-AgP was no more than 3 mm, and the BI of these sites was no more than 2. The comparison of the SCFA concentrations at these sites with those in healthy controls demonstrated that propionic acid and isovaleric acid were still higher in patients with G-AgP ($p < 0.05$). However, there was no obvious difference of formic acid, succinic acid, acetic acid and butyric acid concentration between the two groups. The details are shown in Table 4.

At 6 mo post-therapy, the reduction of probing depth at 59 sites in patients with G-AgP was > 2 mm. In these 59 sites, the reduction of succinic acid, acetic acid, propionic acid and butyric acid concentrations were significantly higher than those at sites where the reduction of probing depth was < 2 mm. The details are shown in Table 5.

The correlation of the presence of subgingival bacteria with the concentrations of short chain fatty acids

At baseline, *P. gingivalis* was detected in 74 of 80 sites, whereas *A. actinomycetemcomitans* was detected only in 14 of 80 sites in patients with G-AgP. The formic acid concentration was higher at sites that were not populated with *F. nucleatum*, *P. gingivalis*, *P. intermedia* and *T. denticola*, compared with sites that contained these microorganisms ($p < 0.05$). By contrast, acetic acid, propionic acid and butyric acid concentrations were significantly higher at sites with *F. nucleatum*, *P. gingivalis*, *P. intermedia* and *T. denticola* compared with those that did not have these microorganisms ($p < 0.05$). No difference in formic acid, acetic acid, propionic acid and butyric acid concentrations was found at sites with or without *A. actinomy-*

Table 4. Comparison of short chain fatty acid concentration in gingival crevicular fluids in the healthy group with PD ≤ 3 mm sites in patients with G-AgP at 2 mo post-treatment

	Healthy group (n = 80)	PD ≤ 3 mm sites in G-AgP patients(n = 36)
Formic acid (mM)	14.0 (10.3–17.9)	12.9 (8.5–22.0)
Succinic acid (mM)	0.3 (0.0–1.5)	0.2 (0.0–0.8)
Acetic acid (mM)	11.3 (6.3–16.2)	10.1 (7.6–16.3)
Lactic acid (mM)	3.9 (2.3–6.3)	5.5 (3.7–9.4)
Propionic acid (mM)	2.1 (1.2–3.1)	3.8 (2.5–6.6)*
Butyric acid (mM)	0.0 (0.0–0.4)	0.0 (0.0–1.1)
Isovaleric acid (mM)	0.0 (0.0–0.9)	0.0 (0.0–1.7)*

G-AgP, generalized aggressive periodontitis; n, number of sites; PD, probing depth.Values are given as medians (lower–upper quartile).
*$p < 0.05$ compared with the healthy group.

Table 3. Concentrations of short chain fatty acids in gingival crevicular fluids of patients with generalized aggressive periodontitis before and after non-surgical periodontal treatment

	Baseline (n = 80)	2 wk (n = 80)	2 mo (n = 80)	4 mo (n = 72)	6 mo (n = 80)
Formic acid (mM)	7.5 (5.4–10.5)	9.5 (6.8–14.6)*	10.2 (7.0–13.5)*	8.8 (6.3–16.1)	9.0 (6.9–12.8)*
Succinic acid (mM)	1.4 (0.8–2.0)	0.4 (0.0–0.9)*	0.5 (0.0–0.9)*	0.8 (0.3–1.3)#	0.5 (0.0–1.1)*
Acetic acid (mM)	26.0 (14.0–38.4)	7.6 (5.9–10.5)*	10.5 (7.5–15.2)*	13.2 (8.9–20.8)*#	10.8 (7.8–17.2)*#
Lactic acid (mM)	8.7 (5.7–11.6)	6.6 (4.5–9.8)	6.7 (3.8–10.4)	8.3 (6.1–11.0)	7.5 (4.7–10.2)
Propionic acid (mM)	8.8 (4.0–12.9)	3.7 (2.0–5.6)*	5.4 (2.9–8.1)	6.6 (4.2–11.2)#	5.2 (2.8–7.2)
Butyrate acid (mM)	2.5 (0.9–4.2)	0.0 (0.0–0.7)*	0.5 (0.0–1.4)*	0.8 (0.0–2.3)*	0.3 (0.0–1.8)*
Isovaleric acid (mM)	2.0 (0.4–3.4)	0.5 (0.0–2.3)*	1.0 (0.0–2.7)*	1.1 (0.0–2.6)*	1.1 (0.0–2.8)#

n, number of sites.Values were given as medians (lower–upper quartile).
*$p < 0.05$, compared with baseline.
#$p < 0.05$, compared with 2-wk post-treatment.

Table 5. Comparison of the reduction in short chain fatty acid concentrations in sites with PD reduction < 2 mm and PD reduction > 2 mm in patients with generalized aggressive periodontitis at 6 mo post-treatment

PD reduction	Formic acid (mM)	Succinic acid (mM)	Acetic acid (mM)	Lactic acid (mM)	Propionic acid (mM)	Butyric acid (mM)	Isovaleric acid (mM)
▵PD < 2 mm (n = 21)	−0.39 (2.7 to −8.2)	0.5 (−0.3 to 0.7)	1.2 (−1.5 to 5.2)	0.3 (−3.1 to 3.8)	−0.3 (−2.1 to 3.1)	0.0 (−0.7 to 1.4)	0.0 (−1.3 to 0.7)
▵PD ≥ 2 mm (n = 59)	−2.4 (0.9 to −4.2)	0.9 (0.37 to 1.41)*	16.1 (5.6 to 27.5)*	1.7 (−3.3 to 5.7)	3.4 (0.1–7.0)*	2.0 (0.6–3.5)*	0.4 (−0.4 to 2.2)

n, number of sites; ▵PD, PD reduction from baseline to 6 mo post-treatment.
Values are given as medians (lower–upper quartile).
*$p < 0.05$, compared with sites where ▵PD < 2 mm.

cetemcomitans. The details are shown in Figs 1–4. The data of succinic acid, lactic acid and isovaleric acid were not shown because of the relatively low concentration and no clear difference between presumed positive or negative sites.

Discussion

This longitudinal study evaluated the effect of non-surgical periodontal treatment on the SCFA levels in gingival crevicular fluid of patients with G-AgP before and after a 6-mo period, as well as SCFA levels in healthy controls. The results showed the effectiveness of non-surgical periodontal treatment on patients with G-AgP, improvement of all clinical parameters were observed after non-surgical periodontal treatment, which was consistent with other studies (4,28–30). Gingival crevicular fluid is an inflammatory exudate that seeps into gingival crevices or periodontal pockets around teeth with inflamed gingiva (31). The analysis of gingival crevicular fluid and subgingival microflora has become increasingly important in the diagnosis and treatment of periodontal diseases. There are several methods of gingival crevicular fluid collection (32), but when collected by paper strips it is suitable for the simultaneous determination of microbial and immunological parameters (33). In this study, SCFAs and periodontal pathogens in the same gingival crevicular fluid sample collected by filter paper strip were analyzed, which make it a possible way to analyze the relationship between the existence of periodontal pathogens and SCFA levels in gingival crevicular fluid. It should be pointed out that *P. gingivalis* was detected in 74 of 80 sites in patients with G-AgP at baseline, followed by *T. denticola* detected in 56 of 80 sites, while *A. actinomycetemcomitans* was only detected in 14 of 80 sites. The results indicate that *A. actinomycetemcomitans* may not be the predominant periodontal pathogens in this group of patients with G-AgP (34).

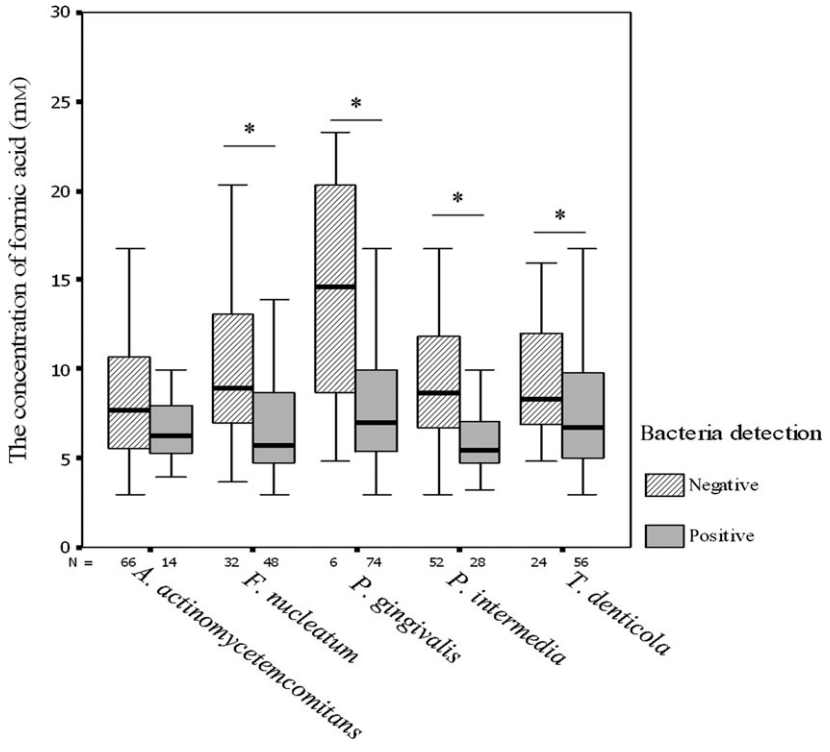

Fig. 1. Comparison of the formic acid concentrations between sites that are positive or negative for *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* at baseline. *$p < 0.05$, compared with bacteria-positive sites. *N*, number of sites.

The post-therapy concentration of formic acid increased significantly, although successful periodontal therapy does not appear to restore SCFA concentrations to the levels seen in health. The formic acid levels in shallow pockets at 2 mo post-treatment were similar to those of healthy controls. This may indicate that the increase of formic acid concentration reflects a tendency to form a microecology beneficial for maintaining periodontal health. This hypothesis was further verified by the observation of lower formic acid concentrations in *P. gingivalis*-, *T. denticola*-,

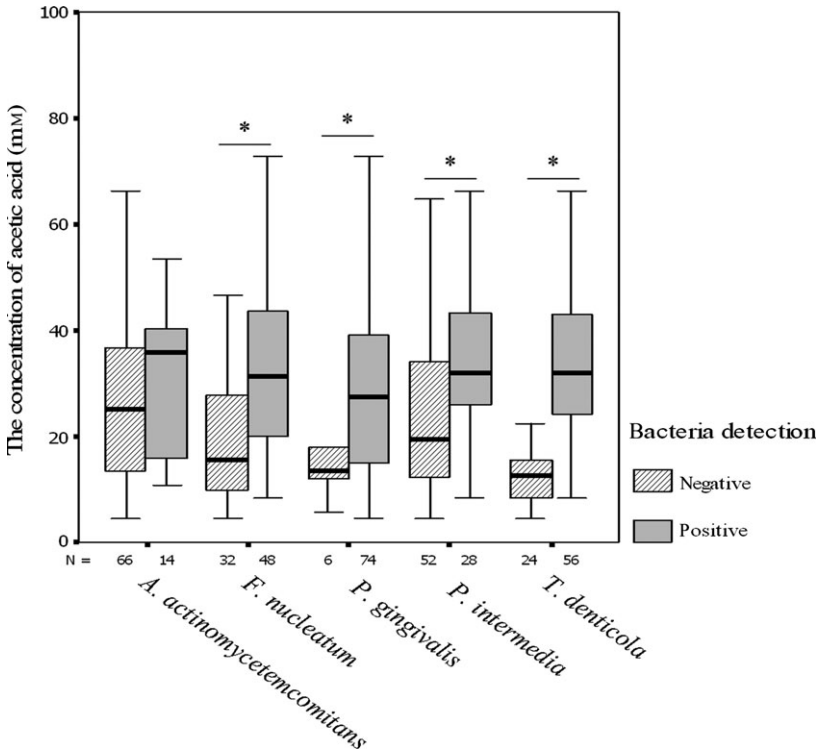

Fig. 2. Comparison of the acetic acid concentrations between sites that are positive and negative for *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* at baseline. $*p < 0.05$, compared with bacteria-negative sites. *N*, number of sites.

P. intermedia- or *F. nucleatum*-positive sites. Our previous studies found that non-surgical periodontal treatment results in a significant reduction of propionic acid, butyric acid and isovaleric acid in gingival crevicular fluids of patients with chronic periodontitis (13). The present findings extend these observations by demonstrating that periodontal therapy significantly decreased succinic acid, acetic acid, propionic acid, butyric acid and isovaleric acid levels in gingival crevicular fluid of patients with G-AgP, all of which reached the lowest level at 2 wk post-treatment. This may be a signal of the change of subgingival micro-ecology after periodontal therapy. However, the patterns of reduction and recolonization of subgingival bacteria over time differed among the different species (30). Repopulation of the subgingival plaque may occur by 120–240 d (35). In this study, it was shown that at 2 mo post-treatment, the concentrations of acetic acid, propionic acid, butyric acid and isovaleric acid started to increase, although at a relatively lower level compared with that of baseline. This may indicate that putative periodontal pathogens start to increase at 2 mo after treatment, and periodontal maintenance may be needed at a relatively short period of 2 mo in patients with G-AgP.

Several hundred recognized species of microorganisms, and many that have yet to be identified, inhabit the gingival crevice (33). SCFA elaboration could contribute to a natural "food web" in oral biofilm resulting in competitive or mutualistic relationships with other oral bacterial co-inhabitants (36). *P. gingivalis* and *P. intermedia* metabolize aspartic acid to succinic acid, but require formic acid as a reducing agent (37). In culture, *P. gingivalis* and *T. denticola* together, the growth of *T. denticola* is stimulated by isobutyric acid generated as a metabolic end-product by *P. gingivalis* (38). However, all the studies mentioned above are *in vitro* studies. The situation *in vivo* is unclear. In the present study, putative periodontal pathogens as well as SCFAs in the same pretreatment gingival crevicular fluid sample of patients with G-AgP were analyzed, the results demonstrated that the concentrations of acetic acid, propionic acid and butyric acid were much higher in sites where *P. gingivalis*, *T. denticola, F. nucleatum* or *P. intermedia* was detected, while the formic acid concentration was lower compared to the negative sites. High levels of acetic acid, propionic acid and butyric acid may indicate putative periodontal pathogen infection. Further studies on bacterial quantification may give more information on the relationship between these bacteria and SCFAs.

It has been proved that not only various subgingival periodontal pathogens produce SCFAs, but SCFAs can also be produced by bacterial fermentation of dietary fiber in the colonic lumen, and present in the systemic circulation. Acetic acid, propionic acid and butyric acid are found at high concentrations in the human intestine (39–41). In the study of Layden *et al*. (8), the total of SCFA concentrations in venous blood of healthy controls are 314 μM, and the concentrations were at their lowest level at fasting in blood (42). Therefore, to control the effect of SCFAs in the circulation on that in gingival crevicular fluid, all the subjects enrolled in this study were physically healthy and were fasting overnight when gingival crevicular fluid was collected. High levels of acetic acid (median: 26.0 mM), propionic acid (median: 8.8 mM), butyric acid (median: 2.5 mM) and isovaleric acid (median: 2.0 mM) were found at baseline in the gingival crevicular fluid of patients with G-AgP, and a significant change of SCFA concentration in gingival crevicular fluid were observed before and after non-surgical periodontal treatment. Therefore, we hypothesize that periodontal bacteria other than SCFAs in the circulation may be the most important factor to influence the SCFA levels in gingival crevicular fluid.

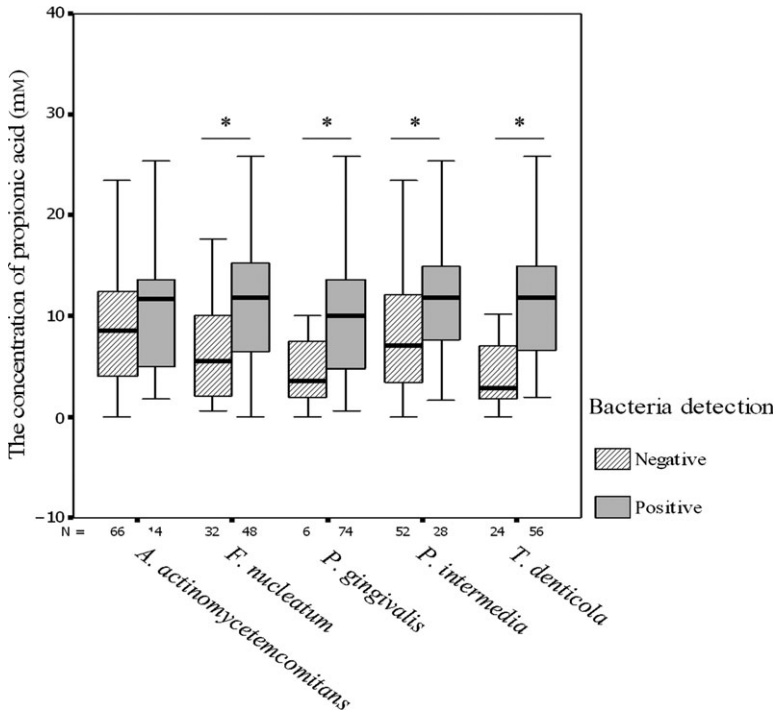

Fig. 3. Comparison of the propionic acid concentrations between sites that are positive and negative for *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* at baseline. $*p < 0.05$, compared with bacteria-negative sites. *N*, number of sites.

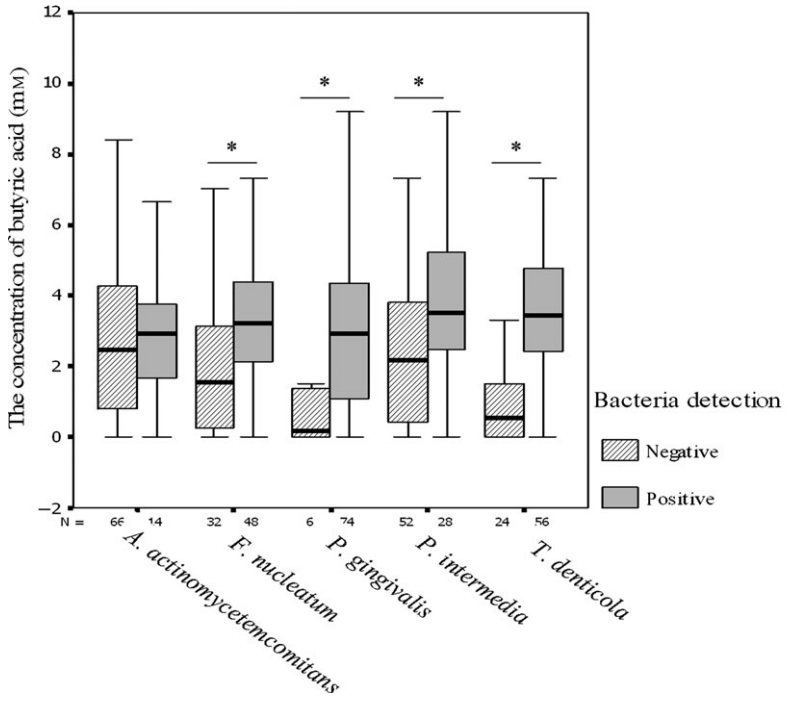

Fig. 4. Comparison of the butyric acid concentration between sites that are positive and negative for *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* at baseline. $*p < 0.05$, compared with bacteria-negative sites. *N*, number of sites.

The concentrations of SCFAs before and after non-surgical periodontal treatment differ significantly; e.g., the concentration of butyric acid is reduced from several mM to nearly 0 mM in gingival crevicular fluids, and the concentration of acetic acid was reduced from over 20 mM to about 10 mM. It is reported that the concentration of butyric acid in the range 0.05–0.1 mM, weakly stimulates cell growth, and at concentrations > 0.2 mM, it inhibits cell proliferation in a dose-dependent manner (18,43). At concentrations > 20 mM, acetic acid can induce interleukin production by peripheral blood mononuclear cells and may be toxic to these cells and neutrophils, whereas at low levels (≤ 2 mM), it does not have such effects (44). SCFAs may play an anti-inflammatory action and in some conditions may play a proinflammatory action, and their specific role in disease pathogenesis and host protection is not known (45). The dramatic changes in SCFA concentrations before and after non-surgical periodontal treatment in patients with G-AgP may have various effects on the destruction and reconstruction of the periodontium, which need further investigation, including the combined effects of the SCFAs on periodontal tissue.

In summary, the present study demonstrated the effect of non-surgical periodontal treatment on levels of SCFAs in gingival crevicular fluid in patients with G-AgP, the formic acid concentration increased with the reduction of periodontal inflammation; this acid may thus be beneficial in maintaining a healthy periodontium. While, the higher concentrations of acetic acid, propionic acid and butyric acid were correlated with periodontal pathogen infection at baseline, the levels of those SCFAs decreased after treatment. SCFA levels may in part be indicators of subgingival microecology; and this area needs further investigation.

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