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ORIGINAL ARTICLE

Full-mouth ultrasonic debridement associated with povidone iodine rinsing in GAgP treatment: a randomised clinical trial

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Abstract
Objective This study evaluated the clinical, immunological and microbiological results of full-mouth ultrasonic debridement (FMUD) with 10 % povidone iodine (PVPI) as the cooling liquid in the treatment of generalised aggressive periodontitis (GAgP).
Material and methods Twenty-eight patients presenting GAgP were randomly assigned to one of the following groups for evaluation: FMUD+SS (n=14)—single session of FMUD with 0.9 % saline solution as cooling agent and FMUD+PVPI (n=14)—single session of FMUD with PVPI solution as cooling agent. Probing depth (PD), relative clinical attachment level (RCAL), relative position of gingival margin, plaque index (FMPI) and bleeding score (FMBS), immunological (interleukin-10 and interleukin-1β concentrations in gingival crevicular fluid) and microbiological (*Aa* and *Pg* amounts) parameters were evaluated at baseline, first, third and sixth months after treatment.
Results The two groups presented reduction of FMPI and FMBS and had statistically significant PD reductions, RCAL gains and gingival recession (p<0.05). Both therapies reduced *Pg* levels in deep and in moderate pockets (p<0.05). FMUD+PVPI reduced *Aa* levels in deep pockets. However, no inter-group differences in clinical, immunological and microbiological parameters were observed (p>0.05).
Conclusions It could be concluded that 10 % PVPI used as an irrigant solution in FMUD decreased *Aa* levels in deep pockets but had no additional benefits when compared with saline solution irrigation in terms of clinical, microbiological and immunological results.
Clinical relevance The FMUD is a valid option for the treatment of GAgP, but the use of 10 % PVPI did not improve the results of the periodontal therapy.

Keywords Aggressive periodontitis · Povidone iodine · Non-surgical periodontal therapy · Full-mouth ultrasonic debridement

Introduction

Generalised aggressive periodontitis (GAgP) is a rare disease occurring in 2.5 to 5 % of the population [1]. However, in spite of its low frequency, its treatment presents a challenge for clinicians, as it presents severe and rapid destruction and a low response to periodontal therapy [2, 3]. Randomised clinical trials focused on achieving more predictable results for GAgP treatment have shown that an antimicrobial approach combined with different non-surgical periodontal treatments (i.e. conventional quadrant scaling and root planing, one-stage full-mouth disinfection and full-mouth ultrasonic debridement) could promote additional benefits. Systemic intake of amoxicillin and metronidazole, adjunctively with non-surgical scaling, has been demonstrated to be the best antimicrobial choice [4–12].

However, in spite of the good results of this combined approach, the literature describes non-adherence by patients to systemic antimicrobial therapy as well as risks of systemic side effects such as nausea, diarrhoea and stomach ache [5].

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Moreover, Guerrero et al. [13] reported that non-adherence to or cessation of antimicrobial therapy negatively influences the results of clinical treatment. In attempts to minimise patient influence on the results of therapy and reduce the systemic side effects of antimicrobial therapy, local antimicrobial therapy seems to be a viable alternative for the treatment of generalised aggressive periodontitis. Systematic review shown additional reduction in probing depth when locally administered adjunctive antimicrobials were used in the periodontal therapy in chronic periodontitis [14]. In this vein, povidone iodine (PVPI) is an interesting alternative, as it has a wide antibacterial spectrum [15–18] and, in vitro, acts rapidly against periodontal pathogens [19]. PVPI also has low financial cost, low systemic toxicity and does not lead to bacterial resistance [20–22].

Periodontal treatment with PVPI as an adjunct to non-surgical periodontal treatment has already been tested in chronic periodontal disease [23]. Systematic and meta-analytical reviews reported minor additional benefit in pocket depth reduction at the third month after treatment with PVPI as an adjunct to conventional scaling and root planing in the treatment of chronic periodontitis [24]. However, this alternative therapy has not yet been tested in GAgP treatment. So, this study tested the hypothesis that there were clinical, microbiological and immunological additional benefits in the use of povidone iodine in the treatment of GAgP patients.

Aim of this study

The aim of this study was to perform a randomised clinical trial to evaluate the clinical, immunological and microbiological results of full-mouth ultrasonic debridement (FMUD) with 10 % povidone iodine solution as a cooling liquid in the treatment of generalised aggressive periodontitis.

Materials and methods

Study design

This study was designed as a parallel, controlled, randomised clinical trial to assess clinical, microbiological and immunological outcomes of FMUD with PVPI as a cooling liquid for the treatment of GAgP. The study was approved by the Ethics Committee of the University of Campinas (024/2006). The first treatment occurred in October 2008, and examinations ended in December 2010.

Study population

The sample evaluated in this study was initially comprised of 34 patients seeking treatment in the Graduate Clinic of Piracicaba Dental School, University of Campinas, Piracicaba, Brazil. The study inclusion criteria were (1) diagnosis of GAgP [2], (2) presence of ≥20 teeth, (3) presence of ≥8 teeth with probing depth (PD) ≥5 mm with bleeding on probing (BOP) and at least 2 teeth with PD ≥7 mm, (4) good general health and (5) <35 years of age. Patients were excluded from the study if they (1) were pregnant or lactating, (2) were suffering from any other systemic diseases (e.g. cardiovascular, diabetes) or conditions affecting periodontal treatment with PVPI, (3) had received antimicrobials in the previous 3 months, (4) were taking long-term anti-inflammatory drugs, (5) had received a course of periodontal treatment within the last 6 months or (6) smoked. Written informed consent was obtained from included participants.

Prior to experimental study, patients underwent 1-month supragingival therapy, which included one session of prophylaxis, supragingival calculus removal and oral hygiene instructions.

Calibration, randomisation and power calculation

Initially, we selected three non-study patients presenting with GAgP. The designated examiner (HFV) measured relative clinical attachment level (RCAL) and PD in all three patients twice within 24 h, with an interval of ≥1 h between examinations. The intraclass correlation coefficient was calculated for each parameter, resulting in 90 % reproducibility for RCALand 91 % for PD.

The randomisation was done by means of an opaque envelope with 34 cards with “PVPI” written on 17 and “saline solution” written on the other 17. Before the treatment, the envelope was opened, and the cooling liquid was revealed. After randomisation, the envelope and lot were discarded. The blinding was broken at the end of the follow-up period.

Sample size calculation was done before the study with statistical software (SAS v.9.1, SAS Institute, Cary, NC, USA). This analysis indicated that with 14 patients in each group, the study would have 80 % power to detect a 1-mm difference in the RCAL of patients in the two groups, considering a SD of 0.8 mm [25].

Periodontal treatment

The patients were randomly assigned to one of two treatment protocols:

1. FMUD+SS (n=17)—one 45-min session of full-mouth ultrasonic subgingival debridement by means of an ultrasonic scaler (Cavitron Select—Dentsply International Inc., Long Island City, NY, USA) and subgingival tips (25 K FSI®-SLI®-10S—Dentsply International Inc.), with 0.9 % saline solution (SS) as a cooling liquid for the ultrasonic device; or

2. FMUD+PVPI (n=17)—the same treatment as in the FMUD+SS group, but the cooling liquid was 10 % povidone iodine (Riodeine—Indústria Farmacêutica Rioquímica Ltda, São José do Rio Preto, SP, Brazil).

Subgingival treatment was performed by the same operator (RCVC), with the patients under local anaesthesia [2 % lidocaine with epinephrine, 1:100,000 (DFL Indústria e Comércio S.A., Rio de Janeiro, RJ, Brazil)]. After the treatment, patients were instructed to take analgesic pills (sodium dipyrone 500 mg, Medley Indústria Farmacêutica Ltda, Campinas, SP, Brazil) for any discomfort inherent to treatment during the first 72 h. Supportive therapy was scheduled, with monthly recalls until the sixth month after treatment. At the first, third and sixth months, clinical, microbiological and immunological evaluations were performed, ending with a session for prophylaxis, supragingival calculus removal and oral hygiene instructions when needed. After the sixth month, teeth that still presented PD ≥5 mm were retreated by conventional scaling and root planing with curettes and local anaesthesia.

Clinical evaluations

Clinical evaluations were assessed before periodontal therapy (baseline) and 1, 3 and 6 months afterward by means of a manual probe (PCPUNC 15®—Hu Friedy, Chicago, IL, USA). The exams were done by the calibrated examiner (HFV), who was blinded until the end of the study.

The following clinical parameters were evaluated: (1) full-mouth plaque index (FMPI), according to Ainamo and Bay [26], and full-mouth bleeding score (FMBS), according to Mühlemann and Son [27]; (2) PD, distance from the bottom of the pocket to the gingival margin; (3) relative gingival margin position (RGMP), distance from the gingival margin to the stent margin and (4) RCAL, distance from the bottom of the pocket to the stent margin.

The measurements were standardised by means of an individually manufactured acrylic stent in which a groove was made to determine the specific site of probing (recorded to the nearest 0.5 mm). All teeth presenting at least one site with PD ≥5 mm were preselected for clinical evaluation, and all presenting pulpal disease or furcation lesions were excluded. From the selected teeth—those presenting more than one site with PD ≥5 mm—only the deepest site was selected. These sites were designated as qualifying sites.

Biofilm and gingival crevicular fluid collection

Subgingival biofilm samples were collected from two sites in each patient, from a pocket presenting 5 mm≤PD<7 mm (moderate pocket) and from another pocket presenting PD ≥7 mm (deep pocket). Following supragingival biofilm removal and relative isolation with cotton rolls, a sterile paper point (#35) (Dentsply Indústria e Comércio, Petrópolis, RJ, Brazil) was inserted into the bottom of the periodontal pocket for 30 s. The paper points were placed in sterile tubes containing 300 μL of 0.5 mM Tris-EDTA.

Gingival crevicular fluid (GCF) was collected from two sites initially presenting 5 mm≤PD<7 mm (moderate pocket) and another two sites presenting PD ≥7 mm (deep pocket). Sixty seconds after subgingival biofilm collection, the area was isolated and gently dried. We collected GCF by placing filter paper strips (Periopaper, OraflowInc, New York, USA) into the pocket until the clinician (HFV) perceived a slight resistance and then leaving them in place for 15 s. This time of 60 s was considered to minimise the chance of contamination of paper strips with blood, but when it occurred, the paper strip was discarded and another sample was collected. The fluid volume was measured with a calibrated electronic GCF measuring device (Periotron 8000, OraflowInc, New York, USA). After volume measurements, the strips were placed in sterile tubes containing 400 mL phosphate-buffered saline (PBS) with 0.05 % polysorbate 20 (Tween 20, Sigma-Aldrich, St. Louis, MO, USA). Both subgingival biofilm and GCF samples were immediately stored at −20 °C.

Microbiological evaluations

The presence and concentrations of *Porphyromonas gingivalis* (*Pg*) and *Aggregatibacter actinomycetemcomitans* (*Aa*) were evaluated with specific primers as reported previously [12, 25], by the real-time polymerase chain reaction (PCR) technique.

Initially, DNA was extracted from the subgingival biofilm. Real-time PCR was performed with the 'hot start' reaction mix for PCR (FastStart DNA Master SYBR Green I, Roche Diagnostics, Mannheim, Germany). The concentration of the DNA used in each run was 10 mg/mL. The amplification profiles were as follows: 95 °C/10 min, 55 °C/5 min and 72 °C/4 min, 40 cycles for *Pg* and 95 °C/10 min, 55 °C/5 min and 72 °C/3 min, 40 cycles for *Aa*. Absolute quantification of target bacteria in clinical samples was performed with *Pg* (ATCC 33277) and *Aa* (JP2) as controls.

The determination of DNA genome copies in controls was based on the genome size of each bacterium and the mean weight of one nucleotide pair. The microbiological analyses were performed separately (moderate and deep pockets).

GCF cytokine levels

Aliquots of each GCF sample were examined by an enzyme-linked immunosorbent assay (ELISA), commercially available in a kit (R&D Systems Inc., Minneapolis, MN, USA) for interleukins IL-1β and IL-10, according to the manufacturer's instructions. Previously, samples were diluted with the

diluent found in the kit. The dilution was considered to calculate the concentration of each GCF substance.

This concentration was calculated with a standard curve, prepared as recommended by the manufacturer. The ELISA assays were run in duplicate, and mean values were used to calculate the concentration of each cytokine. The immunological analyses were performed separately in moderate and deep pockets.

Statistical analysis

Statistical analysis was performed by a professional (GMBA) not involved with the treatment and only for patients who had completed the follow-up (per the protocol). The null hypothesis tested was that FMUD with povidone iodine added no clinical, microbiological or immunological benefits to the treatment of GAgP patients compared with the use of FMUD with saline solution.

The homogeneity of groups at baseline (age, FMPI, FMBS, PD, RCAL and RGMP) was tested by Student's *t* test. For clinical, microbiological and immunological variables presenting normality by the Shapiro-Wilk test ($p>0.05$), repeated-measures ANOVA was used to detect intra- and inter-group differences, with the patient as the statistical unit. When a statistically significant difference was found, an analysis of that difference was determined by the Tukey test.

Variables that did not presented normality were analysed by the Friedman test to detect intra-group differences and by the Mann-Whitney test to detect inter-group differences. The experimental level of significance for all tests was determined to be 5 %. The software used included BioEstat 5.0 (Instituto Sustentável Mamirauá, Belém, PA, Brazil) and SAS 9.1 (The SAS Institute, Cary, NC, USA).

Results

In total, 1280 patients were examined in the Periodontal Clinic of Piracicaba Dental School during the study, but only 34 patients met the inclusion criteria for this study. After the first examination, 34 patients were selected to initiate the protocols, and all were randomly allocated to one of two groups (Fig. 1). In each group, 3 patients were excluded from the analysis, and the follow-up period ended with 28 patients: 14 in the FMUD+SS solution group and 14 in the FMUD+PVPI group.

Table 1 shows the initial demographical and clinical characteristics of subjects included in this study, confirming the homogeneity between groups. Statistically significant differences were not seen when parameters like age ($p=0.98$) and gender ($p=0.38$) and initial values of FMPI ($p=0.78$), FMBS ($p=0.71$), RCAL ($p=0.44$), PD ($p=0.29$) and RGMP ($p=0.37$) were compared between groups.

During the follow-up period, FMPI and FMBS were statistically reduced in the first month and were maintained until the sixth month (Fig. 2). Differences between groups were not seen during the evaluation.

Changes in post-therapy clinical parameters are given in Table 2. PD reduction ($p<0.0001$), RCAL gain ($p<0.0001$) and increase in RGMP ($p<0.0001$) were noted only in the first month after therapy in both groups and were maintained until the sixth month, with no difference between groups concerning RCAL, PD and RGMP at any period of evaluation ($p>0.99$).

When the analyses were made with moderate pockets and deep pockets considered separately, it was seen that both groups promoted RCAL gains, PD reduction and increases in RGMP (Table 3). In both pocket strata, no between-group differences were seen ($p>0.05$). In moderate pockets, PD reduction and RCAL gain after 6 months were 1.35 and 0.76 mm in FMUD+SS ($p<0.0001$) and 1.30 and 0.54 mm in FMUD+PVPI ($p<0.0001$). In deep pockets, PD reduction and RCAL gain after 6 months were 2.74 and 1.75 mm in FMUD+SS ($p<0.0001$) and 2.26 and 1.13 mm in FMUD+PVPI ($p<0.0001$).

In terms of the immunological parameters, in moderate pockets, the two groups did not present IL-1β reductions (Table 4) compared with baseline levels ($p>0.05$), but in deep pockets, the FMUD+SS group presented statisctically reduction of IL-1β in the first month ($p=0.0095$), and these lower levels were maintained until the sixth month ($p=0.0092$). No statistically difference was observed between groups regarding IL-1β levels. The IL-10 levels showed a tendency to increase after therapy (Table 4). For moderate pockets of FMUD+SS group, after 3 months, statistically significantly higher levels were noted than those observed at baseline ($p=0.005$), but at sixth month, these values were not different from baseline ($p=0.8751$). For FMUD+PVPI, no differences in IL-10 levels were observed nor in moderate nor in deep pockets. In spite of that, there were no between-group differences at any period of evaluation ($p>0.05$) (Table 4).

Microbiological analysis (Table 5) showed that *Aa* levels were reduced in FMUD+SS group 3 months after therapy in moderate pockets ($p=0.05$), and no statistically significant difference was seen between groups at any time ($p>0.05$). In deep pockets, *Aa* levels did not present significant reductions in the FMUD+SS group during the follow-up period ($p=0.06$), but in the FMUD+PVPI group, statistical reduction was seen after 3 and 6 months of follow-up ($p<0.05$). No significant differences were observed between groups ($p>0.05$).

There were significant reductions in *Pg* levels at the third and sixth months of follow-up ($p<0.05$), in moderate as well as in deep pockets. However, no significant between-group differences in *Pg* levels were seen ($p>0.05$).

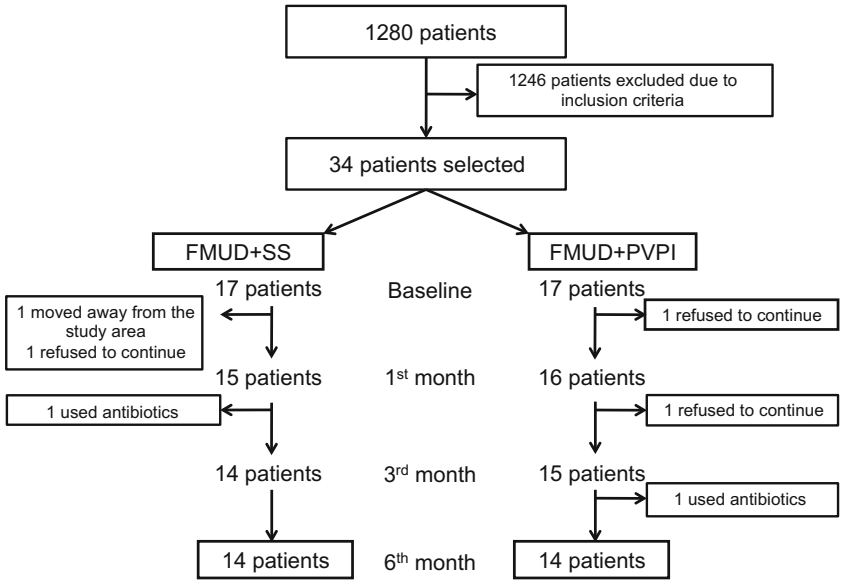

Fig. 1 Flowchart of patients included in the study protocol

Discussion

Aggressive periodontitis has an intricate etiopathology, and many factors are not completely understood [28]. However, in addition to the inconclusive understanding of its pathogenesis, GAgP also presents a reduced response to periodontal treatment [3], making this disease challenging to treat. Different treatment protocols for this disease have been tested [4–12, 29–33], and the most predictable outcomes have been obtained when antimicrobials were associated with mechanical treatment to treat patients with GAgP. Therefore, this study aimed to evaluate the effect of povidone iodine used as a cooling liquid for an ultrasonic scaler in full-mouth ultrasonic debridement.

Few clinical trials have been reported to use povidone iodine in periodontal therapy [23, 34–39]. Those studies evaluated patients with chronic periodontitis treated by different PVPI concentrations and showed discrete benefits when PVPI was associated with traditional periodontal therapy. In comparisons of scaling and root planing (SRP) plus povidone iodine with SRP alone, an additional benefit in PD reduction was seen when povidone iodine was used (0.28 mm, IC 95 %=0.08–0.48 mm, p=0.007) after 6 months of follow-up [24]. Sahrmann et al. [24] observed better clinical results with povidone iodine rinsing and two scaling and root planing interventions, performed at

Table 1 Initial demographical and clinical characteristics of subjects included in each group

Parameters	Treatment		p value
	FMUD+SS (n=14)	FMUD+PVPI (n=14)	
Age (years)	28.57 (±4.59)	28.54 (±4.14)	0.98
Gender F/M	9/5	12/2	0.38
FMPI (%)	29.12 (±11.19)	28.06 (±8.89)	0.78
FMBS (%)	40.93 (±23.06)	43.98 (±18.97)	0.71
RGMP (mm)	2.47 (±1.01)	2.15 (±0.78)	0.37
PD (mm)	6.24 (±0.43)	6.12 (±0.33)	0.29
RCAL (mm)	8.71 (±0.91)	8.36 (±0.79)	0.44

The gender parameter was evaluated by Fisher's exact test. The other parameters were analysed by Student's t test

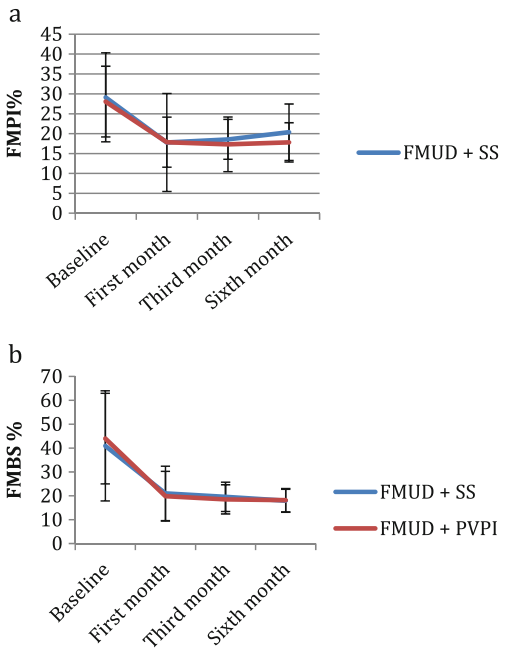

Fig. 2 Mean values (±SD) of full-mouth plaque index (FMPI) (**a**) and full-mouth bleeding score (FMBS) (**b**) of the FMUD+SS (n=14) and FMUD+PVPI (n=14) groups during the 6-month follow-up period. In FMPI as in FMBS, statistically significant differences were evaluated by ANOVA followed by Tukey's test (α=0.05). No inter-group differences were observed (p>0.05). In both groups, the parameters were statistically reduced after the first month compared with baseline (p<0.05)

Table 2 Comparison between groups based on clinical parameters (mean±SD) evaluated: relative gingival margin position (RGMP), relative clinical attachment level (RCAL) and probing depth (PD) during follow-up

Parameters/group	Follow-up period			
	Baseline	First month	Third month	Sixth month
RGMP (mm)				
FMUD+SS (n=14)	2.47 (±1.01) Ab	3.17 (±0.84) Aa	3.34 (±0.98) Aa	3.38 (±0.82) Aa
FMUD+PVPI (n=14)	2.15 (±0.78) Ab	3.13 (±0.69) Aa	3.13 (±0.68) Aa	3.12 (±0.70) Aa
RCAL (mm)				
FMUD+SS (n=14)	8.71 (±0.91) Aa	7.56 (±1.00) Ab	7.38 (±1.00) Ab	7.53 (±0.79) Ab
FMUD+PVPI (n=14)	8.36 (±0.79) Aa	7.91 (±0.72) Ab	7.69 (±0.96) Ab	7.63 (±0.95) Ab
PD (mm)				
FMUD+SS (n=14)	6.24 (±0.43) Aa	4.39 (±0.54) Ab	4.04 (±0.50) Ab	4.19 (±0.65) Ab
FMUD+PVPI (n=14)	6.12 (±0.33) Aa	4.78 (±0.47) Ab	4.56 (±0.56) Ab	4.51 (±0.49) Ab

Data from all qualifying sites are given

Different lowercase letters in rows and *different uppercase letters* in columns indicate statistically significant differences by repeated-measures ANOVA followed by Tukey's test (p<0.05)

least 1 month apart. Recently, Sahrmann et al. [39] presented that additional PD reduction was obtained 3 months after scaling and root planing with PVPI gel as adjunct substance. The authors suggested that the gel formula has a longer substantivity than the PVPI solution; therefore, it would remain for a longer period of time inside the periodontal pocket, consequently improving the clinical results. Differently from both studies previously mentioned, in the present study, the PVPI solution was only used during the initial debridment with the reinstrumentation of residual pockets performed after 6 months. The use of PVPI solution did not promote additional clinical benefits.

Table 3 Comparison between groups based on clinical parameters (median (interquartile ranges)) evaluated, including relative gingival margin position (RGMP), relative clinical attachment level (RCAL) and probing depth (PD) of moderate (5 mm≤PD<7 mm) and deep (PD≥7 mm) pockets

Initial pocket strata	Parameter/group	Follow-up period		
		Baseline	Third month	Sixth month
Moderate pocket	RGMP (mm)			
	FMUD+SS	2.92 (2.33–3.25) Ab	3.42 (2.67–3.92) Aa	3.25 (3.17–3.75) Aa
	FMUD+PVPI	2.13 (1.63–2.89) Ab	2.98 (2.44–3.54) Aa	2.90 (2.53–3.51) Aa
	RCAL (mm)			
	FMUD+SS	8.00 (7.83–8.57) Aa	7.25 (6.67–7.58) Ab	7.17 (6.50–8.00) Ab
	FMUD+PVPI	7.54 (7.29–8.30) Aa	7.13 (6.73–7.84) Ab	7.13 (6.58–7.78) Ab
	PD (mm)			
	FMUD+SS	5.36 (5.00–5.50) Aa	3.50 (3.28–4.33) Ab	3.57 (3.25–4.83) Ab
	FMUD+PVPI	5.50 (5.25–5.62) Aa	4.00 (3.83–4.52) Ab	4.00 (3.79–4.68) Ab
Deep pocket	RGMP (mm)			
	FMUD+SS	2.33 (1.28–3.00) Ab	2.83 (2.20–4.25) Aa	3.30 (2.20–4.00) Aa
	FMUD+PVPI	2.00 (1.75–2.50) Ab	3.00 (2.75–3.50) Aa	3.06 (2.75–3.50) Aa
	RCAL (mm)			
	FMUD+SS	9.38 (8.57–10.00) Aa	7.25 (6.71–8.33) Ab	7.25 (6.58–8.50) Ab
	FMUD+PVPI	9.00 (8.90–10.18) Aa	8.06 (7.58–9.28) Ab	8.31 (7.68–9.00) Ab
	PD (mm)			
	FMUD+SS	7.29 (7.20–7.50) Aa	4.50 (4.14–5.00) Ab	4.67 (4.07–5.00) Ab
	FMUD+PVPI	7.25 (7.03–7.45) Aa	5.31 (4.56–5.87) Ab	5.06 (4.62–5.50) Ab

For the parameters moderate RCAL and deep RGMP, *different lowercase letters* in rows indicate a statistically significant difference by the Friedman test (p<0.05), and *different uppercase letters* in columns indicate a statistically significant difference by the Mann-Whitney test (p<0.05). For the other parameters, different *lowercase letters* in rows and *different uppercase letters* in columns indicate statistically significant differences by repeated-measures ANOVA followed by Tukey's test (p<0.05)

Table 4 Comparison between groups based on immunological variables (mean±SD) evaluated: interleukin-1 beta and interleukin 10 of moderate (5 mm≤PD<7 mm) and deep (PD≥7 mm) pockets

Initial pocket strata	Parameter/group	Follow-up period			
		Baseline	First month	Third month	Sixth month
Moderate pockets	IL-1β (pg/mL)				
	FMUD+SS	33.55±25.64 Aa	22.00±14.38 Aa	20.23±15.19 Aa	20.51±20.97 Aa
	FMUD+PVPI	25.00±18.71 Aa	10.05±6.23 Aa	11.06±8.09 Aa	12.76±8.1) Aa
	IL-10 (pg/mL)				
	FMUD+SS	0.22±0.36 Ab	0.47±0.75 Aab	1.59±2.67 Aa	0.52±0.87 Aab
	FMUD+PVPI	0.32±0.66 Aa	0.71±0.75 Aa	0.80±0.91 Aa	0.85±1.04 Aa
Deep pockets	IL-1β (pg/mL)				
	FMUD+SS	53.21±39.10 Aa	20.41±12.02 Ab	25.79±25.84 Ab	19.68±20.43 Ab
	FMUD+PVPI	30.44±23.33 Aa	12.39±9.83 Aa	12.40±9.26 Aa	18.67±8.14 Aa
	IL-10 (pg/mL)				
	FMUD+SS	0.08±0.14 Aa	0.29±0.50 Aa	0.44±0.59 Aa	0.27±0.45 Aa
	FMUD+PVPI	0.05±0.07 Aa	0.31±0.37 Aa	0.39±0.40 Aa	0.31±0.41 Aa

Different lowercase letters in rows and *different uppercase letters* in columns indicate statistically significant differences by repeated-measures ANOVA followed by Tukey's test ($p<0.05$)

Using the proposed protocol of the present study, povidone iodine presented in moderate pockets a RCAL gain of 0.54±0.52 mm and PD reduction of 1.30±0.47 mm (while in control group, these values were, respectively, 0.76±1.15 and 1.35±1.13 mm). In deep pockets, the RCAL gains were 1.75±0.98 and 1.13±0.94 mm, and PD reductions were 2.74±0.75 and 2.26±0.98 mm in the FMUD+SS and FMUD+PVPI groups, respectively. This absence of additional clinical benefits of subgingival irrigation has been described by Krück et al. [38], who treated patients with chronic periodontitis by scaling and root planing, followed immediately by pocket irrigation with 0.9 % sodium chloride solution, 0.12 % chlorhexidine digluconate or 7.5 % povidone iodine. This study showed no statistical difference between the irrigation substances regarding PD reduction, clinical attachment level gain and bleeding on probing reduction. Koshy et al. [40] and Zanatta et al. [23], using the same non-surgical therapy protocol with an ultrasonic device and povidone iodine or water irrigation to

Table 5 Between-group comparisons based on microbiological variables: quantitative presence (median (interquartile ranges)) of *Aggregactibacter actinomycetemcomitans* (*Aa*) and *Porphyromonas gingivalis* (*Pg*) in moderate (5 mm≤PD<7 mm) and deep (PD≥7 mm) pockets

Initial pocket strata	Parameter/group	Follow-up period		
		Baseline	Third month	Sixth month
Moderate pocket	*Aa**			
	FMUD+SS	5.46 (3.96–5.99) Aa	2.04 (0–4.60) Ab	4.12 (0.75–4.95) Aab
	FMUD+PVPI	5.04 (4.69–5.78) Aa	3.76 (2.44–4.23) Aa	4.13 (1.43–5.26) Aa
	*Pg**			
	FMUD+SS	5.82 (5.49–7.25) Aa	1.19 (0–4.82) Ab	0 (0–4.20) Ab
	FMUD+PVPI	5.42 (2.42–5.85) Aa	0.49 (0–2.20) Ab	0 (0–1.65) Ab
Deep pocket	*Aa***			
	FMUD+SS	5.49 (0–5.72) Aa	3.30 (0–5.15) Aa	3.38 (0–4.34) Aa
	FMUD+PVPI	5.33 (3.97–5.47) Aa	3.77 (1.92–4.18) Ab	3.45 (1.47–3.93) Ab
	*Pg**			
	FMUD+SS	5.83 (5.15–6.74) Aa	0 (0–2.90) Ab	2.21 (0–4.02) Ab
	FMUD+PVPI	4.87 (4.33–5.62) Aa	2.25 (0–3.61) Ab	1.10 (0–3.12) Ab

**Different lowercase letters* in rows and *different uppercase letters* in columns indicate statistically significant differences by repeated-measures ANOVA followed by Tukey's test ($p<0.05$)

***Different lowercase letters* in rows indicate statistically significant differences by the Friedman test ($\alpha=0.05$), and *different uppercase letters* in columns indicate statistically significant differences by the Mann-Whitney test ($\alpha=0.05$)

treat generalised chronic periodontitis, also failed to found additional clinical benefits with PVPI irrigation.

The FMUD+PVPI group presented PD reduction and RCAL gain of 1.30 and 0.54 mm in moderate pockets after 6 months. In deep pockets, the values of PD reduction and RCAL gain after 6 months were 2.26 and 1.13 mm in that group. Studies investigating the association of scaling and root planing with systemic antibiotics (amoxicillin+metronidazole) found PD reductions and clinical attachment gains of 1.4 and 1.6 [7] and 2 and 2 mm [11] in moderate pockets. In deep pockets, the same studies found PD reductions and clinical attachment gains of 3.7 and 4.2 mm [7] and 4.0 and 3 mm. Although the present study did not investigate the comparison between topical and systemic antibiotics, it seems plausible that the concentrations of antibiotics in gingival crevicular fluid after periodontal therapy could explain the lower values found in the present study. During systemic antibiotic therapy, the concentrations of drugs in gingival crevicular fluid are greater than in a single topical use of PVPI, which, in deep pockets, could probably reduce the bacterial challenge, thereby aiding the host response.

The values found in this study were similar to those found in controlled groups of some studies that tested GAgP treatments. Guerrero et al. [5] presented, in the control group (scaling and root planing+placebo), PD reductions of 1.0 and 1.8 mm, and clinical attachment gains of 0.8 and 1.3 mm in moderate and deep pockets, respectively. Hughes et al. [41] showed, in deep pockets, a PD reduction of 2.11±2.01 mm and a clinical attachment gain of 1.77±2.15 mm after scaling and root planing. Similar values were also found by Haas et al. [9] in moderate (PD reduction of 1.25±0.17 mm and clinical attachment gain of 0.74±0.28 mm) and deep pockets (PD reduction of 2.76±0.51 mm and clinical attachment gain of 1.35±0.34 mm) in patients treated by scaling and root planing plus placebo. Mestnik et al. [11] showed PD reductions and clinical attachment gains of approximately 1.0 and 1.0 mm in moderate pockets and 2.50 and 2.00 mm in deep pockets 3 months post-scaling and root planing alone. The results found in this study are in agreement with those presented in the literature, supporting the idea that the protocol used in this study could be indicated for the treatment of GAgP.

After treatment, there was an increase on the relative position of the gingival margin values on all groups; however, no difference was observed between the groups. In spite of this increase, one cannot infer that gingival recession occurred, since this parameter was represented by the distance from the gingival margin until the stent used during the clinical examinations and not the cement-enamel junction. Instead, these results indicate that both therapies were able to reduce the gingival margin edema, which is aimed during periodontal therapy.

Concerning the immunological parameters, IL-1β is a pro-inflammatory cytokine, and its level could be used as a bone loss predictor in patients with periodontitis [42]. Thus, the reduction in IL-1β is desirable after periodontal therapy. For the anti-inflammatory cytokine IL-10, one of its functions is the suppression of tissue destruction [43], which makes its increase after treatment an expected goal of therapy [44]. In this study, both groups promoted this tendency to reduce IL-1β and no difference was observed between groups. Difference was found only in deep pockets for FMUD+SS group. Tendency to increase IL-10 levels were also seen in both groups, but no differences in intra- or inter-group evaluations were observed. Other authors who evaluated these inflammatory markers also presented high inter-individual variability of GCF markers and have not shown statistically significant differences between groups with chronic [25] and GAgP disease [12, 45, 46].

On the microbiological evaluation, povidone iodine therapy did not promote statistical reduction of *Aa* levels in moderate pockets, and no statistically significant difference was seen between groups during the evaluation period. Interestingly, in deep pockets, the FMUD+PVPI group showed a statistically significant reduction in *Aa* levels on the 3-month evaluation. These results are in agreement with Krück et al. [38] who showed that PVPI could exert some effect on *Aa* reduction 3 months after the therapy. Nonetheless, in the present study, both groups remained with similar outcomes and no statistical difference. Although no difference could be seen between groups, *Aa* is a key pathogen in GAgP disease and should be a focus of disease control, as its presence has been associated with further clinical attachment loss. It has been shown to persist in periodontal sites when these were treated only by mechanical therapy, with significant reductions when antimicrobials were adjunctively used [12].

Regarding the *Pg* level, no significant additional reduction could be noticed with the use of PVPI. Studies that have presented additional benefits of PVPI use in the reduction of *Pg* levels [38, 39] performed different treatment protocols. Krück et al. [38] found significant *Pg* reduction using one session full-mouth scaling and root planing with curretes and ultrasonic device instrumentation 5 min per tooth associated with 10 mL povidone iodine irrigation per quadrant, and the patients were instructed to rinse their mouth twice daily with 10 mL of 0.12 % chlorhexidine digluconate for 10 days. Sahrmann et al. [39] found lower levels of *Pg* in the PVPI group compared to the baseline values, the treatment protocol included scaling and root planing associated with PVPI gel application. Sahrmann et al. [47] also presented that povidone iodine gel seems to be a good way to use this farmacol being detectable in the pocket 15 min after the application, allowing a prolonged remnant effect in the pocket.

In the present study, the FMUD protocol could also negatively influence PVPI action in the subgingival region, as, in this protocol, all teeth were instrumented within 45 min,

which represents less than 2 min per tooth. It has been reported in the literature that PVPI acts against periodontal bacteria in a very low time exposure [19], but Sahrmann et al. [24] described that factors like GCF and bleeding can dilute and wash the PVPI from the periodontal pocket, negating its effective action. Sahrmann et al. [47] described that povidone iodine solution applied immediately after scaling and root planing presents significant concentration decrease within 1 min, which is probably influenced by the bleeding and GCF. The use of specific subgingival ultrasonic inserts seems to be insufficient for spraying the irrigation solution all the way to the bottom of the periodontal pocket [48]. Differently from the use of a syringe, in the ultrasonic insert, the liquid was released at the middle of the insert. This characteristic could influence the extent of PVPI irrigation inside the pocket, which might have affected its antimicrobial action.

Since this is the first study enrolling generalised aggressive periodontitis subjects treated by povidine iodine adjuctive to mechanical therapy, there are still answer to be searched for. Thus, future studies including new treatment approaches should consider including this hard-to-treat population and assess the benefits of PVPI in this kind of patients. Also, different methods of PVPI application should be tested to evaluate its effect on microbiological and, consequently, clinical/immunological results.

Conclusion

We concluded that 10 % PVPI used as an irrigant solution in FMUD decreased *Aa* levels in deep pockets but had no additional benefits when compared with saline solution irrigation in terms of clinical, microbiological and immunological results.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures in this study were performed in accordance with the ethical standards of the Ethics Committee of Piracicaba Dental School (protocol 024/2006), and written informed consent was obtained from all individual participants included in the study.

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