Acta Odontologica Scandinavica, 2007; 65: 52–59

informa
healthcare

ORIGINAL ARTICLE

Microbiological effect of the use of an ultrasonic device and iodine irrigation in patients with severe chronic periodontal disease: A randomized controlled clinical study

ÅSA LEONHARDT[1], CHRISTINA BERGSTRÖM[2], LENA KROK[3] & GIUSEPPE CARDAROPOLI[4]

[1]*Department of Periodontology, Specialist Dental Clinic, Mölndal Hospital, Mölndal, Sweden and Department of Oral Microbiology, Faculty of Odontology, Sahlgrenska Academy of Göteborg University, Göteborg, Sweden,* [2]*Artimplant AB, Västra Frölunda, Sweden,* [3]*Department of Periodontology, Specialist Dental Clinic, Mölndal Hospital, Mölndal, Sweden,* [4]*Faculty of Odontology, Sahlgrenska Academy at Göteborg University, Göteborg, Sweden and Department of Periodontology, University of Siena, Siena, Italy*

Abstract

Objective. Instrumentation of the subgingival area is aimed at removing as much as possible of the bacterial biofilm and subgingival calculus. Since mechanical root debridement is a technically demanding procedure, antiseptics and antibiotics delivered either locally or systemically have been used as adjunct to scaling and root-planning procedures in order to control the subgingival biofilm and thereby enhance the treatment outcome. Our aim was to study the microbiological effect of ultrasonic debridement with or without povidone-iodine (PVP-iodine) in the treatment of severe chronic periodontitis. ***Material and Methods.*** Twenty patients were recruited to the study. Each test site and the related quadrant were randomly assigned to one of four different treatment modalities: ultrasonic scaling+subgingival irrigation with 0.5% PVP-iodine for 5 min/tooth, ultrasonic scaling+subgingival irrigation with sterile saline solution for 5 min/tooth, subgingival irrigation with sterile saline solution for 5 min/tooth and subgingival irrigation with 0.5% PVP-iodine for 5 min/tooth. The individuals were followed longitudinally for 6 months. ***Results.*** The present study showed that non-surgical periodontal therapy with the use of an ultrasonic device was effective in reducing the analyzed putative periodontal bacteria. No statistically significant difference between ultrasonic+saline and ultrasonic+PVP-iodine was found. ***Conclusions.*** Ultrasonic debridement reduced the periodontal markers in patients with severe chronic periodontitis. The reduction was selective. A concentration of 0.5% PVP-iodine did not add any anti-microbiological effect compared to ultrasonic debridement alone.

Key Words: *Iodine, microbiological effect, periodontal treatment, ultrasonic debridement*

Introduction

Since the role of micro-organisms in the causation and pathogenesis of periodontal disease is well documented, therapy is directed primarily at reducing the number of pathogenic micro-organisms, however unspecifically. Several hundred recognized species inhabit the gingival crevice [1], but it has been shown that only a few play any significant role in the etiology of periodontal diseases [2]. *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Treponema denticola* are considered putative periodontal pathogens. The presence of these pathogens has been suggested as promoting an increased risk of further periodontal tissue breakdown.

Mechanical removal of the subgingival biofilm and calculus is considered essential for controlling periodontal disease. The mechanical effect of various subgingival debridement procedures has shown that a wide range of approximately 5–80% of treated roots harbor residual plaque or calculus deposits. Scaling efficacy is reduced with increasing pocket depth and furcation involvement, and the deeper the pockets and furcation involvement, the more deposits are left behind [3]. Since thorough subgingival scaling is a technically demanding procedure,

Correspondence: Åsa Leonhardt, Department of Periodontology, Specialist Dental Clinic, Mölndal Hospital, SE-431 80 Mölndal, Sweden. Tel: +46 31 86 15 00. Fax: +46 31 86 15 01. E-mail: asa.leonhardt@vgregion.se

(Received 20 March 2006; accepted 18 August 2006)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350600973078

applied antiseptics and antibiotics delivered either locally or systemically have been used as adjunct to scaling and root-planning procedures in order to control the subgingival biofilm and thereby enhance the treatment outcome. The results presented in the literature, however, are inconclusive [4,5].

Povidone-iodine (PVP-iodine) is a water-soluble combination of polyvinylpyrolidone and iodine [6]. It has a bactericidal effect and is effective against most bacteria, including putative periodontal pathogens [7]. Short-term or long-term exposure to PVP-iodine has been used in the treatment of periodontal disease together with ultrasonics to enhance the treatment outcome [8–11]. The effect reported in these studies, however, was limited or inconclusive. In a longitudinal study on ultrasonic debridement with or without PVP-iodine by Leonhardt et al. [12], indirect parameters such as bleeding on probing and probing pocket depth were analyzed to evaluate the treatment outcome.

The aim of the present study was to evaluate the direct effect of the treatment procedures, i.e. the microbiological effect of ultrasonic debridement with or without PVP-iodine in the treatment of severe chronic periodontitis.

The null hypothesis tested was that the use of PVP-iodine in conjunction with mechanical root debridement does not reduce/eliminate bacterial markers in patients with severe periodontal disease.

Material and methods

Subjects

Twenty patients (8 M, 12 F) aged 39–68 years at baseline (mean age 54 years) referred for treatment of advanced periodontal disease and fulfilling the inclusion criteria were recruited to the study.

Individuals with at least 1 single-rooted tooth/quadrant with at least 1 site showing probing pocket depth (PPD) $\geq$6 mm and bleeding on probing (BoP) after a period of 1 month plaque control obtaining presence of plaque (P) $\leq$15% were included. Exclusion criteria included allergy to iodine, thyroid dysfunction, and requirement of antibiotic prophylaxis prior to dental treatment or systemic antibiotic treatment within the preceding 3 months, pregnancy, diabetes or any medical condition comprising a contraindication for routine dental treatment. Smokers were not included in the study. The protocol was approved by the Ethics Board at Sahlgrenska Academy, Göteborg University and written informed consent was obtained from each study object.

Periodontal treatment

Following the pre-examination (visit before baseline), each subject was given information about their periodontal condition and recommended treatment. After that they received a thorough supragingival scaling and were instructed to have proper plaque control with the use of toothbrush and interdental brushes [13]. When plaque index $\leq$15% was obtained, the treatment started, i.e. at baseline. In each subject, four test sites, each located in single rooted teeth and in different quadrants, were selected based on having probing pocket depth of 6 mm or greater. By means of a sealed envelope drawing, each test site and the related quadrant were randomly assigned to one of four different treatment modalities: 1) Ultrasonic scaling (Odontogain®, XO-CARE A/S, Denmark), 42 000 Hz + subgingival irrigation with 0.5% PVP-iodine for 5 min/tooth. 2) Ultrasonic scaling (Odontogain®, XO-CARE A/S, Denmark), 42 000 Hz + subgingival irrigation with saline for 5 min/tooth. 3) Subgingival irrigation with saline for 5 min/tooth. 4) Subgingival irrigation with 0.5% PVP-iodine for 5 min/tooth.

The treatment procedure, carried out by an experienced dental hygienist (L.K.), was performed in 2 quadrants (1 and 4) at the same time, and the other week in the other two quadrants (2 and 3). Quadrants receiving mechanical debridement were treated under local anesthesia performed with Xylocain adrenaline 2% (Astra AB, Mölndal, Sweden).

Clinical examination

Examinations of plaque score (P), probing pocket depth (PPD), and bleeding on probing (BoP) were performed at the pre-examination. Plaque score was calculated as percentage surfaces positive for plaque (presence/absence). PPD measurements were recorded parallel to the long axis of the tooth using a 1-mm scaled periodontal probe (North Carolina PCP-15, Hu-Friedy, Chicago, Ill., USA) at six location points around the circumference of each tooth as the distance between the gingival margin and the bottom of the probable pocket to the nearest whole millimeter. In conjunction with measuring probing depth, the area was observed for presence/absence of bleeding on probing (BoP).

In each subject, 1 single-rooted tooth/quadrant was selected which had at least 1 approximal pocket with probing depth of $\geq$6 mm. Among the selected sites, all had a suprabony location. The selected sites were exposed to further assessments at baseline, 3 and 6 months. The following parameters were recorded: plaque score, probing pocket depth, bleeding on probing, probing attachment level (PAL) using a standardized periodontal probe and the cemento-enamel junction as a reference point. All measurements were produced by one blinded investigator (Å.L.). The clinical data are presented elsewhere [12].

Bacterial sampling

Subgingival samples (1 from the deepest site/study tooth) for bacterial analysis were performed at baseline, 1 week, 3 months, and 6 months for examination of the presence of 18 subgingival species. This was done using the "checkerboard" DNA-DNA hybridization methodology according to Socransky et al. [14]. Diseased sites (1/quadrant) were isolated with cotton rolls and supra-gingival plaque was removed with sterile cotton pellets. One sterile paper point/tooth (Johnson and Johnson, East Windsor, N.J., USA) inserted to the depth of the periodontal pocket and kept in place for 15 s was analyzed using the checkerboard technique [15].

Checkerboard DNA-DNA hybridization

The presence and level of 18 subgingival species were determined using the "checkerboard" DNA-DNA hybridization methodology [14,15]. Briefly, the samples were boiled for 5 min, neutralized, laid down in lanes on nylon membranes (Roche Diagnostics Scandinavia AB, Bromma, Sweden) using a MiniSlot device (Immunetics, Cambridge, Mass., USA) and immobilized by UV-light and incubated at 120°C over night. In a Miniblotter apparatus, 18 digoxygenenin-labeled whole genomic DNA probes were placed in lanes vertically to the plaque samples. After 2 h of pre-hybridization, the DNA probes were allowed to hybridize over night at 42°C. After being stringently washed at 65°C, hybrids were detected by means of a phosphatase conjugated anti-digoxigenin antibody and signals were visualized in a Lumi Imager workstation.

Evaluation of the number of bacteria in the samples was performed by comparing the obtained signals with the signals generated by pooled standard samples containing 10^6 and 10^5 of each of the species. The signals were coded on a scale from 0 to 5, score 0 indicating no signal; score 1 signal-density weaker than the one of the low standard (i.e. $<10^5$ bacteria); score 2 signal-density equal to the one of the low standard (10^5 = bacteria); score 3 signal-density higher than the one of the low standard but lower than that of the high standard ($>10^5$ but $<10^6$ bacteria); score 4 signal-density equal to the one of the high standard ($>=10^6$ bacteria); and score 5 signal-density higher than the one of the high standard ($>10^6$ bacteria). The species analyzed were: *P. gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *T. forsythensis*, *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, *T. denticola*, *Micromonas micros*, *Campylobacter rectus*, *Eikenella corrodens*, *Selenomonas noxia*, *Streptococcus intermedia*, *Streptococcus oralis*, *Streptococcus sanguis*, *Streptococcus mutans*, *Veillonella parvula*, and *Actinomyces naeslundii*. All assessments were performed by one single examiner.

Statistics

One tooth per quadrant from each individual was used as registration site in the present study and descriptive statistics were used for presentation of registered data. The frequencies of positive sites regarding the different bacterial species were statistically evaluated. Chi-square tests were used to analyze the differences between the different treatment groups. The Cochran test and the McNemar test were used to evaluate the differences between the different follow-up periods. The Bonferroni method was used to adjust the p-values [16].

Results

All results regarding the clinical parameters have been reported elsewhere [12]. Ultrasonic debridement with saline or PVP-iodine gave a reduction in the number of positive individuals regarding putative periodontal pathogens (Figures 1 and 2). Non-periopathogens such as streptococci and *A. naeslundii* were only influenced marginally (Figure 2).

The percentage positive individuals at the different time intervals, from baseline, 1 week, 3 and up to 6 months are shown in Figures 1 and 2. A pronounced trend of decrease was seen in the number of individuals positive for the analyzed bacteria in the teeth treated with ultrasonic+saline and ultrasonic+iodine 1 week after baseline, i.e. after therapy. At 3 months, several of the analyzed species had re-growth to almost baseline values (Figure 1). For some species, i.e. *P. gingivalis*, *P. nigrescens*, and *T. forsythensis* there was a decrease in the number of positive individuals also at 6 months (Figure 1). No obvious decrease could be seen after therapy for *S. oralis* and *S. mutans* (Figure 2). After treatment with ultrasonic+saline, a significant difference was seen between baseline and 6 months ($p<0.01$) for *P. intermedia*, between baseline and 3 months ($p<0.05$) for *P. micros* and between baseline and 6 months ($p<0.01$) for *A. naeslundii.* After treatment with ultrasonic+saline, a significant difference was seen between baseline and 1 week ($p<0.01$), baseline and 3 months ($p<0.01$), and baseline and 6 months ($p<0.05$) for *T. forsythensis* between baseline and 3 months ($p<0.01$) and baseline and 6 months ($p<0.05$) for *P. micros*, and between baseline and 6 months ($p<0.01$) for *A. naeslundii.* With ultrasonic+iodine a significant difference was seen between baseline and 6 months ($p<0.05$) for *V. parvula* and between baseline and 6 months ($p<0.01$) for *A. naeslundii*. Ultrasonic+iodine resulted in a significant difference between baseline and 6 months ($p<0.01$) for *A. naeslundii* and between baseline and 3 months ($p<0.05$) for *M. micros*. A significant difference was seen for *T. forsythensis* at 1 week, 3 months, and 6 months, and for *F. nucleatum* at 3 months when comparing

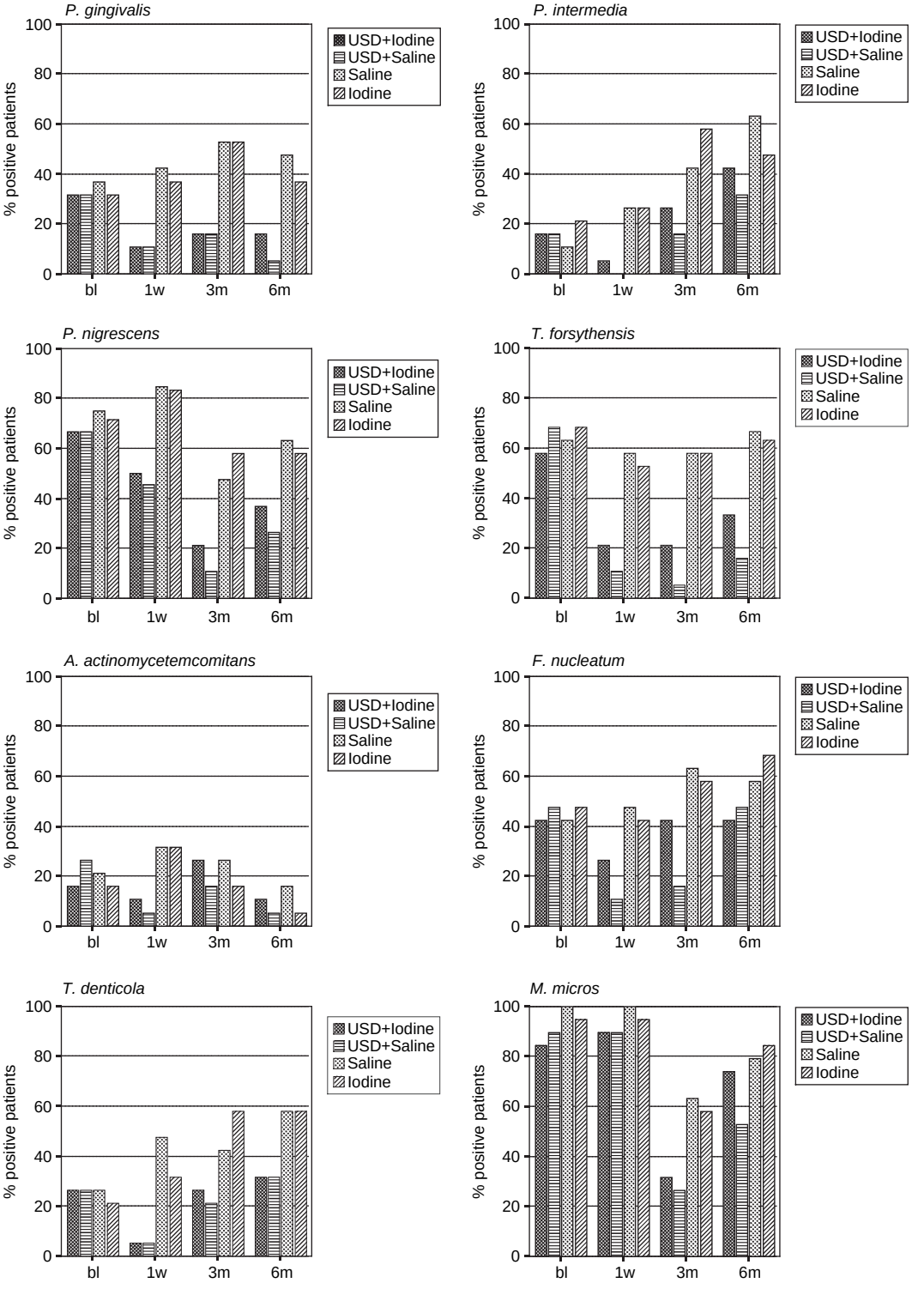

Figure 1. Percentage positive individuals in the different treatment groups for the analyzed bacteria, $n = 20$ (bl = baseline).

ultrasonic + saline with saline irrigation ($p < 0.05$). When comparing ultrasonic + saline with iodine, a significant difference was seen for *P. intermedia* at 3 months ($p < 0.05$) and for *T. forsythensis* at 3 and 6 months ($p < 0.05$). *S. noxia* and *C. ochraea* were not presented in a figure since they occurred in few individuals and followed the same pattern as the others. The pattern for irrigation groups 3 and 4 was that no obvious decrease occurred in percentage positive individuals compared to baseline (Figures 1 and 2).

Discussion

The aim of the study was to evaluate the microbiological effect of ultrasonic debridement with or without PVP-iodine in the treatment of severe chronic periodontitis. This 6 months follow-up study

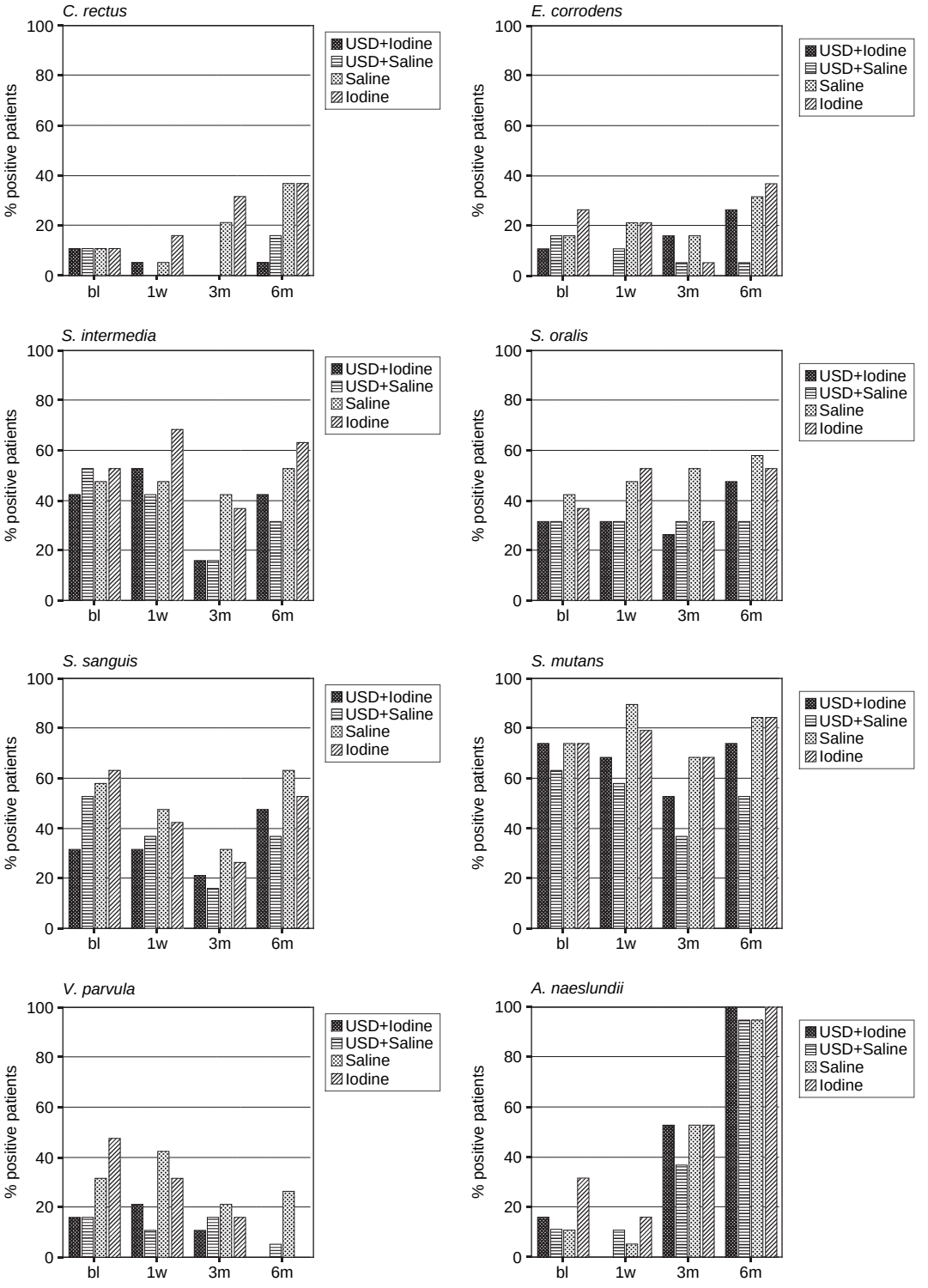

Figure 2. Percentage positive individuals in the different treatment groups for the analyzed bacteria, $n = 20$ (bl = baseline).

showed that the addition of PVP-iodine did not reduce/eliminate the analyzed bacterial markers more than the ultrasonic debridement alone, therefore the null hypothesis is accepted.

In the present study, non-surgical periodontal therapy performed by means of ultrasonic instrumentation plus the use of PVP-iodine or saline resulted in reduction of the analyzed periodontal pathogens. However, the reduction was selective. No statistically significant difference was found between the two ultrasonic groups. For various reasons, some patients do not respond favorably to conventional mechanical therapy alone. The use of an adjunctive antimicrobial might benefit this subset of patients. Earlier studies have examined the additional antimicrobial effect of PVP-iodine in combination with subgingival debridement [6]. In some small clinical trials, thorough debridement along with application

of PVP-iodine enhanced the effect of non-surgical periodontal therapy [8–10]. The results from these studies, however, suggest that the addition of certain antimicrobials to the lavage during ultrasonic instrumentation were of minimal clinical benefit. In a recent publication by Del Peloso Ribeiro [11] it was reported that the use of PVP-iodine as an adjunct to subgingival instrumentation did not provide additional benefits in Class II furcation defects.

An important factor in the treatment of periodontal disease is removal of the biofilm from the tooth surface [17]. One of the abilities of biofilm is protection from chemical products, for example PVP-iodine [18]. Substantivity of an antimicrobial system implies the ability of that system to maintain adequate antimicrobial drug levels over a sufficient period of time. It is accepted that the amount of antimicrobial necessary to affect the residents of a biofilm is several orders of magnitude greater than the amount required to inhibit planktonic bacteria [17]. Another explanation for why we could not find any additive effect of iodine could be that a too low concentration was used. Quirynen et al. [4] proposed that a repeated application of PVP-iodine at a 10% concentration was necessary to obtain an effect. Cargill et al. [19] have shown that legionellae in biofilms were 135 times more resistant to iodine compared to micro-organisms growing in a non-organized or planktonic fashion. Recently, Slots [20] recommended subgingival irrigation of 10% PVP-iodine by a syringe for 5 min to control subgingival colonization of periodontal pathogens. In the present study, 0.5% PVP-iodine for 5 min was used. Furthermore, to be effective, antimicrobials must reach their target site and be maintained there at sufficient concentrations long enough for their antimicrobial effect to occur. Earlier studies have shown that the antibacterial activity of iodine is of short duration [21,22]. In the present study, each tooth was treated for 5 min. Iodine may also be negatively influenced by biological factors such as dentin matrix, type-I collagen [23]. Möller [22] has stated that iodine was readily inactivated by organic substances in endodontic infections.

Non-surgical mechanical treatment, which includes mechanical plaque control, scaling, and root planning, is the first recommended step and is an indispensable phase of periodontal therapy. The outcome of periodontal treatment is based on supra- and subgingival plaque control. This is assured thorough oral hygiene to remove supragingival plaque deposits and by meticulous scaling during subgingival debridement. With subgingival debridement, it is possible to reduce the amount of bacteria [3], reduce pocket depths and improve clinical attachment levels [24], which assures effective treatment of chronic periodontitis and long-term stability. The clinical outcome of the present study as presented elsewhere [12] confirms that non-surgical treatment, performed by means of an ultrasonic device, was effective in reducing probing pocket depth. This is in accordance with the published literature [25]. Conventional non-surgical periodontal therapy consists of mechanical supra- and subgingival tooth debridement and instruction in self-administered oral health-care measures. These measures are directed towards reducing the bacterial load and altering the microbial composition towards a flora more associated with health. The present study showed that all putative periodontal pathogens of the species analyzed decreased after therapy. However, they were not completely eliminated, not even reduced, under the detection level. The reduction was selective, i.e. the Gram-negative periodontopathogens, e.g. *P. gingivalis*, *P. intermedia*, *T. forsythensis*, and *T. denticola* were markedly reduced, while the Gram-positive bacteria, such as streptococci and Actinomyces, did not seem to be affected at all. Other studies have shown that despite large reduction an eradication of all bacteria seems hard to reach. Several explanations have been presented, such as that bacteria can reside in soft tissues [26,27] or in root surface irregularities and dentinal tubules [28]. The mechanical procedures undoubtedly removed most organisms that colonized the tooth surface. Consequently, if periodontopathogenic bacteria are only partly eliminated from the periodontal pocket, re-growth will occur. Given the rapid multiplication rates of bacteria it is not surprising that the majority of the analyzed pathogens returned to almost baseline levels at 3 months. The present study showed that ultrasonic treatment reduced the periodontal pathogens, while the percent positive sites with streptococci seem unchanged, i.e. the microbial composition was altered towards a flora associated with healthy conditions. Following removal of the bacterial biofilm, parts of the early colonizers, which are primarily non-pathogenic, may be faster in occupying the "vacant" habitat and thus inhibit the establishment of pathogens. A recent study by Rhemrev et al. [29] reported that subgingival cleaning in itself had limited effect in actually removing bacteria. Previous studies have shown that re-growth of the subgingival area by micro-organisms may occur within 2–8 weeks after treatment [30,31]. Other data in the literature suggest that the return to baseline total counts might occur within 4–8 days [32]. The present study showed that for several species the change in the microflora is temporary, i.e. 1–3 months, except for *P. gingivalis*, which was reduced at 6 months. The time needed for re-growth to reach full pretreatment levels of the subgingival microbiota depends on the severity of the periodontal disease and of the thoroughness of debridement with subsequent supportive therapy. The fact that the periodontal pathogens were not eliminated

below detection level may have had an impact on the outcome of the periodontal treatment and should perhaps be evaluated by sampling instead of by register plaque index and pocket depth to determine whether the treatment was successful or not.

Ecological niches for bacteria, other than periodontal pockets, within the oral cavity are unlikely to be affected by scaling, as oral mucous membranes, tongue dorsum, and saliva may constitute a source for re-growth [33]. Earlier studies have shown that supragingival plaque plays an important part in the re-growth process [30,31,34]. Plaque development is shown to be dependent of periodontal inflammation [35,36]. The present study had a period of plaque control before baseline and the individuals were followed-up regarding plaque level during the 6-month period [12].

The conclusion from the present study is that ultrasonic debridement reduced the periodontal markers, but did not eliminate them, in patients with severe chronic periodontitis. A concentration of 0.5% PVP-iodine did not add any microbiological effect to the ultrasonic debridement alone.

References

[1] Moore WEC, Moore LVH. The bacteria of periodontal disease. Periodontol 2000 1994;5:66–77.

[2] Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134–44.

[3] Petersilika GI, Ehmke B, Flemmig TF. Antimicrobial effects of mechanical debridement. Periodontol 2000 2002;28:56–71.

[4] Quirynen M, Teughels W, De Soete M, Van Steenberghe D. Topical antiseptics and antibiotics in the initial therapy of chronic adult periodontitis: microbiological aspects. Periodontol 2000 2002;28:72–90.

[5] Slots J, Ting M. Review. Systemic antibiotics in the treatment of periodontal disease. Periodontol 2000 2002;28: 106–76.

[6] Greenstein G. Povidone-iodine effects and role in the management of periodontal diseases: a review. J Periodontol 1999;70:1397–405.

[7] Caufield PW, Allen DN, Childen NK. In vitro susceptibilities of suspected periodontopathic anaerobes as determined by membrane transfer assay. Antimicrob Agents Chemother 1987;31:1989–93.

[8] Rosling BG, Slots J, Christersson LA, Gröndahl H-G, Genco RJ. Topical antimicrobial therapy and diagnosis of subgingival bacteria in the management of inflammatory periodontal disease. J Clin Periodontol 1986;13:975–81.

[9] Christersson LA, Rosling BG, Dunford RG, Wikesjö UM, Zambon JJ, Genco RJ. Monitoring of subgingival Bacteroides gingivalis and Actinobacillus actinomycetemcomitans in the management of advanced periodontitis. Adv Dent Res 1988;2:382–8.

[10] Forabosco A, Baletti R, Spinato S, Colao P, Casalari C. A comparative study of a surgical method and scaling and root planing using the Odontoson. J Clin Periodontol 1996;23: 611–4.

[11] Del Peloso Ribeiro E, Bittencourt S, Bovi Ambrosano GM, Notici FH, Sallum EA, Sallum AW, et al. Povidone-iodine used as adjunct to non-surgical treatment of furcation involvements. J Periodontol 2006;77:211–7.

[12] Leonhardt Å, Bergström C, Krok L, Cardaropoli G. Healing following ultrasonic debridement and PVP-iodine in individuals with severe chronic periodontal disease. A randomized controlled clinical study. Acta Odontol Scand 2006;64: 262–6.

[13] Axelsson P, Lindhe J. The significance of maintenance care in the treatment of periodontal disease. J Clin Periodontol 1981;8:281–94.

[14] Socransky SS, Smith C, Martin L, Paster BJ, Dewhirst FE, Levin AE. "Checkerboard" DNA-DNA hybridization. Biotechniques 1994;17:788–92.

[15] Papapanou PN, Madianos PN, Dahlén G, Sandros J. "Checkerboard" versus culture: a comparison between two methods for identification of subgingival microbiota. Eur J Oral Sciences 1997;106:389–96.

[16] Lehmann EL. Statistical methods based on ranks. In: Nonparametrics. New York: Holden-Day; 1975. p. 5–43, 266–81

[17] Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontol 2000 2002;28:12–55.

[18] Marsh PD, Bradshaw DJ. Physiological approaches to the control of oral biofilms. Adv Dent Res 1997;11:176–85.

[19] Cargill KL, Pyle BH, Sauer RL, Mcfeters GA. Effects of culture conditions and biofilm formation on the iodine susceptibility of Legionella pneumophilia. Can J Microbiol 1992;38:423–9.

[20] Slots J. Selection of antimicrobial agents in periodontal therapy. J Periodont Res 2002;37:389–98.

[21] Engström B, Hård Segerstad L, Ramström G, Frostell G. Correlation of positive cultures with the prognosis for root canal treatment. Odont Revy 1964;15:257–70.

[22] Möller ÅJR. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Odontol Tidskr 1966 Dec 20;74(5): Suppl 1–380.

[23] Portenier I, Haapasalo H, Örstavik D, Yamauchi M, Haapasalo M. Inactivation of the antibacterial activity of iodine potassium iodide and chlorhexidine digluconate against enterococcus faecalis by dentin, dentin matrix, type-I collagen, and heat killed microbial whole cells. J Endod 2002;28:634–7.

[24] van der Weijden GA, Timmerman MF. A systematic review on the clinical efficacy of subgingival debridement in the treatment of chronic periodontitis. J Clin Periodontol 2002; 27(Suppl 3):55–71.

[25] Badersten A, Nilveus R, Egelberg J. Effect of nonsurgical periodontal therapy II. Severely advanced periodontitis. J Clin Periodontol 1984;11:63–76.

[26] Cugini MA, Haffajee AD, Smith C, Kent RL, Socransky SS. The effect of scaling and root planning on the clinical and microbiological parameters of periodontal diseases: 12 months results. J Clin Periodontol 2000;27:30–6.

[27] Flemmig TF, Milian E, Karch H, Klaiber B. Differential clinical treatment outcome after systemic metronidazole and amoxicillin in patients harboring Actinobacillus actinomycetemcomitans and/or Porphyromonas gingivalis. J Clin Periodontol 1998;25:380–7.

[28] Adriaens PA, Edwards CA, De Boever JA, Loesche WJ. Ultrastructural observations on bacterial invasion in cementum and radicular dentin of periodontally diseased human teeth. J Periodontol 1988;59:493–503.

[29] Rhemrev GE, Timmerman MF, Veldkamp I, van Winkelhoff AJ, Van der Velden U. Immediate effect of instrumentation on the subgingival microflora in deep inflamed pockets under strict plaque control. J Clin Periodontol 2006;33: 42–8.

[30] Magnusson I, Lindhe J, Yoneyama T, Liljenberg B. Recolonization of a subgingival microbiota following scaling in deep pockets. J Clin Periodontol 1984;11:193–207.

[31] van Winkelhoff AJ, van der Velden U, De Graaff J. Microbial succession in recolonizing deep pockets after a single course of supra- and subgingival debridement. J Clin Periodontol 1988;15:116–22.

[32] Furuichi Y, Ramberg P, Krok L, Lindhe J. Short-term effects of triclosan on healing following subgingival scaling. J Clin Periodontol 1997;24:777–82.

[33] Van Steenberghe TJM, van der Velden U, Abbas F, De Graaf J. Microflora and bacterial DNA restriction analysis in young adults with periodontitis. J Periodontol 1991;62: 235–41.

[34] Sbordone L, Ramaglia L, Gulletta E, Iacono V. Recolonization of the subgingival microflora after scaling and root planing in human periodontitis. J Periodontol 1990;61:579–84.

[35] Goh CJ, Waite IM, Groves BJ, Cornick DE. The influence of gingival inflammation and pocketing on the rate of plaque formation during non-surgical periodontal treatment. Br Dent J 1986;161:165–9.

[36] Ramberg P, Lindhe J, Dahlén G, Volpe AR. The influence of gingival inflammation on de novo plaque formation. J Clin Periodontol 1994;21:51–6.