J Clin Periodontol 2002: 29: 540–550
Printed in Denmark . All rights reserved

Journal of Clinical Periodontology
0303-6979

Local antimicrobial therapy after initial periodontal treatment

A randomized clinical trial comparing three biodegradable sustained release polymers

Giovanni E. Salvi[1], Andrea Mombelli[2], Lisa Mayfield[1], Alexandra Rutar[1], Jean Suvan[4], Steven Garrett[3] and Niklaus P. Lang[1]

[1]University of Berne, School of Dental Medicine, Berne, [2]University of Geneva, School of Dental Medicine, Geneva, Switzerland; [3]Atrix Laboratories Inc., Fort Collins, Colorado, USA, [4]Eastman Dental Institute, University College London, London, UK

Salvi GE, Mombelli A, Mayfield L, Rutar A, Suvan J, Garrett S, Lang NP. Local antimicrobial therapy after initial periodontal treatment. A randomized clinical trial comparing three biodegradable sustained release polymers. J Clin Periodontol 2002; 29: 540–550. ©Blackwell Munksgaard, 2002.

Abstract

Aim: The aim of this single-blind, randomized, parallel-designed clinical trial (RCT) was to evaluate the clinical and microbiological effects of three sustained-release biodegradable polymers delivered into periodontal pockets following initial periodontal therapy.

Methods: Forty-seven patients (28 females and 19 males) with a mean age of 51 years (range 29–71) underwent a periodontal examination at baseline (i.e. Week 0) and after 18 weeks. This included the assessment of the Plaque Index (PlI), Bleeding on Probing (BOP), Pocket Probing Depths (PPD) and Probing Attachment Levels (PAL) at six sites per tooth. Two to 4 months prior to baseline, all subjects had received initial periodontal therapy including motivation, instruction in oral hygiene practices and full-mouth scaling and root planing. At the treatment appointment (i.e. Week 2), the patients were randomly assigned to receive either Atridox™, Elyzol® Dental Gel or PerioChip® at all residual periodontal pockets with a probing depth ≥5 mm and concomitant BOP. In accordance with the manufacturer's recommendations, Elyzol® Dental Gel was applied for a second time 7 days later. In addition to the clinical evaluation, subgingival microbiological samples were collected prior to treatment (i.e. Week 2) and at Weeks 4 and 18. Analysis of variance/covariance was used to evaluate changes from baseline to Week 18 for the clinical parameters.

Results: Between the baseline and 18-week examinations, subjects treated with Atridox showed a significantly greater gain in mean PAL of 0.33 mm ± 0.09 (SD) than subjects treated with Elyzol® Dental Gel [0.03 mm ± 0.09 (SD)] ($p = 0.03$). However, the gain in PAL of 0.16 mm ± 0.10 (SD) found after PerioChip® application did not differ significantly from that obtained following the application of Atridox™ ($p = 0.27$). Of the sites treated with Atridox™, 42% gained ≥1 mm PAL and 9% ≥2 mm PAL as opposed to the sites treated with Elyzol® Dental Gel, in which 34% gained ≥1 mm PAL and 8% gained ≥2 mm PAL. Of the sites treated with PerioChip®, 36% gained ≥1 mm and 6% gained ≥2 mm PAL following a completed initial periodontal therapy.

Conclusions: The application of the three biodegradable sustained release devices tested following initial periodontal therapy resulted in a statistically significant gain in mean PAL for Atridox™ and a significant reduction in PPD for all three devices during the study period. Furthermore, when sites treated with Atridox™ were compared with sites treated with Elyzol®, a significant difference in mean PAL gain (0.3 mm) was observed.

Key words: biodegradable drug delivery systems; microbiological effects; periodontal disease; periodontal therapy; randomized clinical trial (RCT)

Accepted for publication 2 July 2001

The presence of bacterial plaque represents the principal etiologic factor involved in the initiation and progression of inflammatory periodontal diseases (for review see Offenbacher 1996 and Zambon 1996). The inflammatory host response resulting from this bacterial infection may lead to progressive destruction of the supporting periodontal structures. Therefore, one of the key elements of periodontal therapy is to achieve a significant reduction or even eradication of suspected periodontal pathogens. Regular and adequate oral hygiene combined with non-surgical mechanical debridement such as scaling and root planing and, in some instances, additional surgical therapy using access flaps have been documented extensively (for review see Palcanis 1996) to successfully arrest the progression of periodontal tissue destruction.

A novel non-mechanical approach aimed at eliminating the subgingival microbiota consists of the administration of local biodegradable sustained-release antimicrobial agents into periodontal pockets. This additional therapy should provide an antimicrobial concentration adequate to penetrate a biofilm in the periodontal pocket for prolonged time periods. Prolonged delivery of an antimicrobial agent substantially above (i.e. 80–100 times) its minimum inhibitory concentration (MIC) *in vitro* is of particular importance because of the organization of the subgingival microbiota as a biofilm.

Several biodegradable local delivery systems containing different antimicrobial agents have recently been developed and introduced to the market as an adjunctive measure to mechanical therapy or even as an independent monotherapy (for review see Greenstein & Polson 1998 and Rams & Slots 1996). Numerous studies have investigated the use of biodegradable sustained-release polymers or gels either during initial periodontal therapy (Ainamo et al. 1992, Garrett et al. 1999, Jeffcoat et al. 1998, Lie et al. 1998, Klinge et al. 1992, Noyan et al. 1997, Pedrazzoli et al. 1992, Polson et al. 1997, Soskolne et al. 1997) or during supportive periodontal therapy (Garrett et al. 2000, Riep et al. 1999, Rudhart et al. 1998, Stelzel & Flores-de-Jacoby 1996, Stelzel & Flores-de-Jacoby 1997). However, no data are available to determine the treatment outcomes of biodegradable sustained-release polymers applied at the time of reevaluation following initial periodontal therapy in sites not satisfactorily responding to treatment. Only a few studies have addressed their use in recurrent or persistent periodontal lesions, a potentially valuable area of application (Kinane & Radvar 1999, Radvar et al. 1996). Therefore, the aim of this study was to evaluate the clinical and microbiological outcomes of three sustained-release biodegradable polymers delivered into periodontal pockets following initial periodontal therapy.

Table 1. Demographic characteristics and smoking history

	Elyzol®	PerioChip®	Atridox™	Total
Males	6	6	7	19
Females	10	9	9	28
Mean age, years	52	54	47	51
Age range	38–65	43–71	29–61	29–71
Never smoked	8	5	8	21
Current smokers	4	5	5	14
Former smokers	4	5	3	12

Material and Methods

Study design

This 4-month, single-center study followed a randomized, parallel, single-blind design to compare the clinical and microbiological results of subgingivally administered (i) 8.5% doxycycline hyclate (Atridox™, Block Drug Corporation, Inc., Jersey City, NJ, USA) (ii) 25% metronidazole benzoate (Elyzol® Dental Gel, Dumex Ltd, Copenhagen, Denmark), and (iii) chlorhexidine gluconate (PerioChip®, Perio Products Ltd, Jerusalem, Israel). The subjects of this study had been diagnosed with chronic periodontitis and had received full-mouth scaling and root planing 2–4 months prior to baseline examination. Subjects between 25 and 75 years of age were included if they had a minimum of two teeth, distributed in the mouth to represent isolated experimental units (i.e. at least one tooth apart), with an approximal residual periodontal pocket of at least 5 mm that bled on probing. Each subject had at least two sites at two separate teeth, fulfilling these criteria. Subjects were not included if they were using any contraindicated medications, presented with compromised systemic conditions, or had received scaling and root planing within the last 2 months. Teeth were not included if the depth of the pocket extended to the apex of the tooth because of possible endodontic/periodontal complications. No peri-implant sites were studied. A total of 51 subjects were entered into the study of which 47 completed the 4-month examination.

Treatment procedures

Each subject was assigned to one of three treatment groups according to a computer-generated randomization code. All sites with a PPD $\geq$ 5 mm were treated. The formulation containing doxycycline hyclate (DH) was a solution containing 8.5% weight to weight doxycycline hyclate, 37% weight to

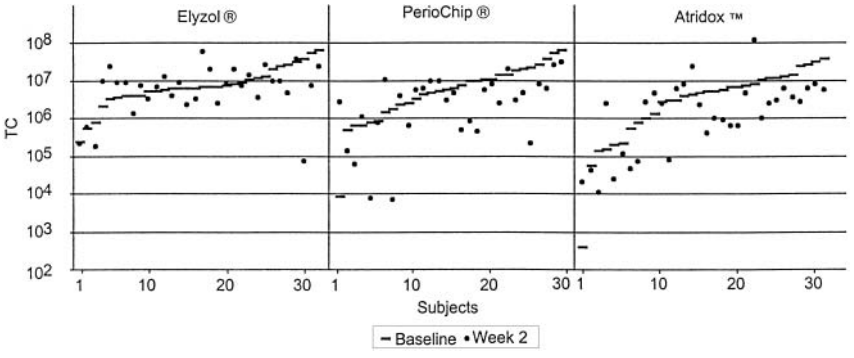


Fig. 1. Comparison of the ETSA total cultivable counts (TC) at baseline and 2 weeks later. Within groups the subjects are sorted by the baseline TCs.

weight poly DL-lactide (PLA) dissolved in a biocompatible carrier of 63% weight to weight N-methyl-2-pyrrolidone (NMP) (Atridox). Atridox is composed of two separate syringes that are coupled together just prior to use and mixed for 100 cycles. Once mixed, Atridox was allowed to sit at room temperature for 15 min and then mixed for another 10 cycles before use. A 23-gauge blunt cannula was attached to the delivery syringe and Atridox was injected into the periodontal pocket. Any overflow material was gently packed into the pocket with a moist instrument in order to speed coagulation of polymer.

Elyzol Dental Gel is an off-white semisolid suspension of 1 g metronidazole benzoate equivalent to 25% metronidazole. A second application of Elyzol Dental Gel was performed 7 days after baseline treatment in accordance with manufacturer's recommendations. All qualifying sites were treated and no periodontal dressing was used.

The PerioChip is a small, bullet-shaped, thin film containing 2.5 mg chlorhexidine gluconate incorporated in a biodegradable matrix of cross-linked hydrolyzed gelatin. After isolating and drying the area, one PerioChip was completely inserted into each periodontal pocket using a pair of forceps.

Evaluation of treatment effect

Clinical parameters were assessed and microbial samples were taken by an investigator who was not involved in treatment and who was unaware of the choice of treatment provided. Clinical parameters were recorded at baseline and 18 weeks after treatment. The periodontal examination included the assessment of Probing Pocket Depth (PPD), Probing Attachment Level (PAL), Bleeding on Probing (BOP) and Plaque Index (PlI) (Silness & Löe 1964) at six sites per tooth using a University of North Carolina (UNC) 15 manual periodontal probe. In addition, a check of adverse events, dental procedures and the use of concomitant medications was performed 2 and 6 weeks after baseline treatment.

The subgingival microbiota was sampled using sterile endodontic paper points at the two predetermined locations at baseline, as well as 2 and 18 weeks after treatment. The samples were transported to the laboratory under anaerobic conditions within 15

Table 2. Frequency of detection at baseline (1), Week 2 (2), and Week 18 (3) of P. gingivalis (PGI), P. intermedia/nigrescens (PIN), P. melaninogenica Group (PMG), B. forsythus (BFO), Fusobacterium sp. (FUS), C. rectus. (CRE), and A. actinomycetemcomitans (AAT). Black fields indicate positive status, each line represents one site

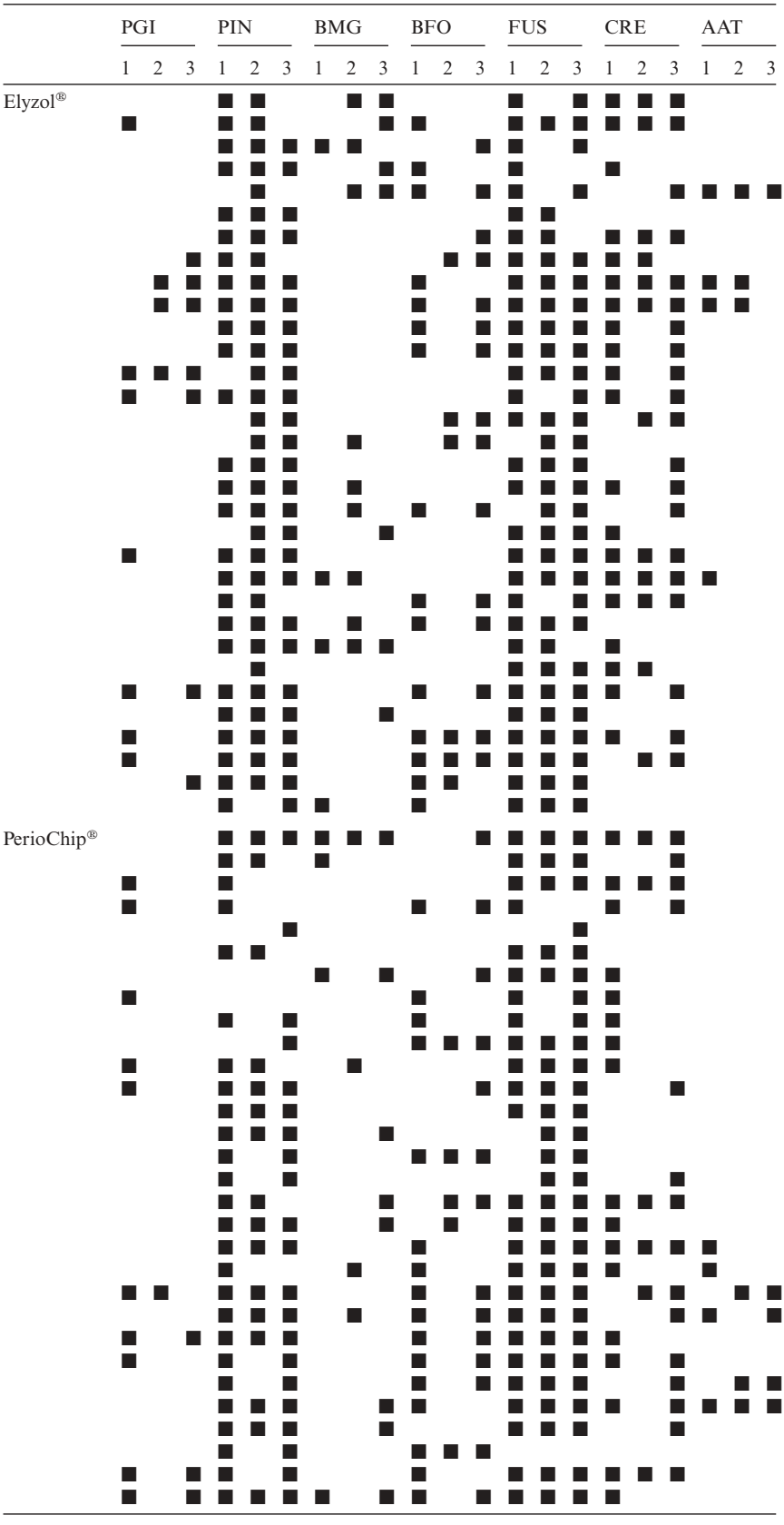
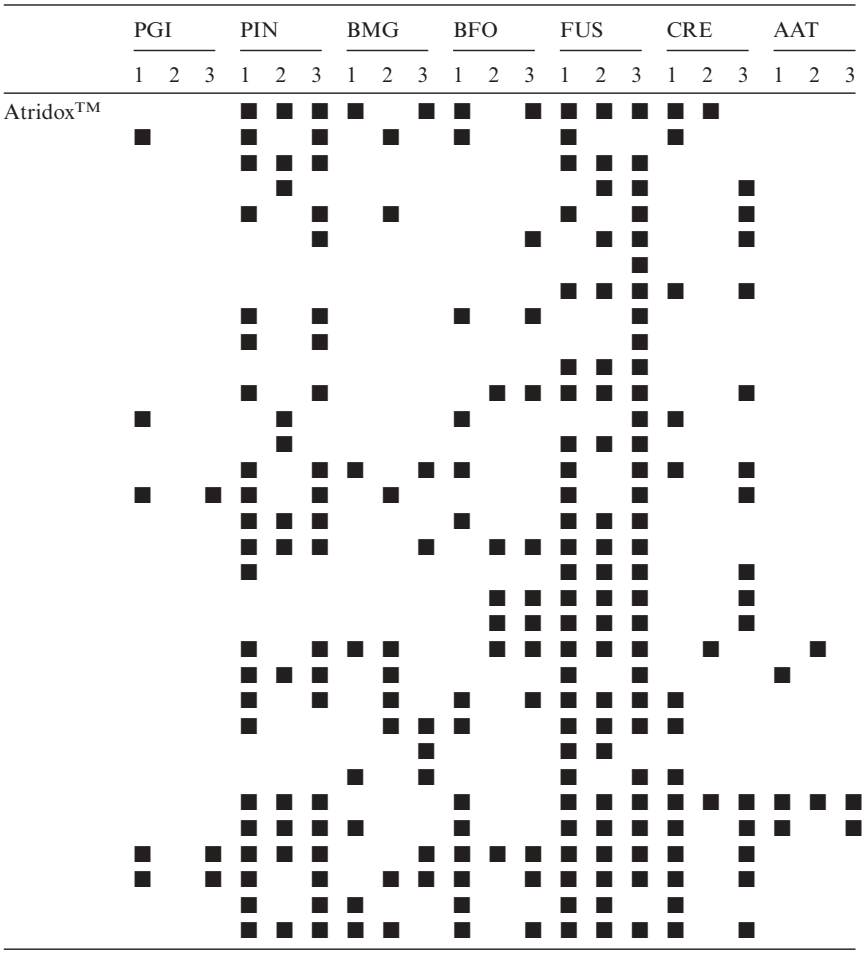


Table 2. Continued



min. They were serially diluted and plated in a standardized way onto Enriched Tryptic Soy Agar (ETSA) (Syed et al. 1980) and Tryptic soy-Serum-Bacitracin-Vancomycin agar (TSBV) (Slots 1982). ETSA plates were anaerobically incubated in an anaerobic chamber, containing an atmosphere of 85% N_2, 10% H_2 and 5% CO_2. TSBV agar plates were incubated in a 5% CO_2 air incubator. Six bacterial taxa were identified from the anaerobic culture based on the Gram-stain, aerotolerance, motility, esculin hydrolysis, nitrate reduction, indole production, α-glucosidase and N-benzoyl-DL-arginine-2-trypsin hydrolysis, oxidase and catalase activities: *Porphyromonas gingivalis*, *Prevotella intermedialnigrescens*, *Prevotella melaninogenica* Group (indole negative black pigmenting Gram-negative bacilli, including *P. melaninogenica*, *P. loescheii* and *P. denticola*), *Bacteroides forsythus*, *Fusobacterium* spp., and *Campylobacter rectus*. On TSBV agar, *Actinobacillus actinomycetemcomitans* was identified based on its colony morphology and a positive catalase test. For the details of the microbiological procedures the reader is referred to Keller et al. (1998).

Data analysis

The primary objectives of this study were assessed using an analysis of variance/covariance to compare the changes in clinical parameters and in total aerobic and anaerobic bacterial counts and specific periodontal pathogens between baseline and Month 4. The null hypothesis was that no difference existed in clinical parameters and total aerobic and anaerobic bacterial counts and specific periodontal pathogens between the three treatment groups. The alternative hypothesis was that a difference existed between the three groups.

The total colony counts on anaerobic ETSA were transformed into Colony Forming Units (CFU) per sample (i.e. per 1 mL transport fluid) using predetermined conversion factors to account for dilution and the size of the evaluated surface on the plate. One colony of a target organism growing on a plate inoculated with the lowest dilution of the sample – the minimal requirement for a positive detection – corresponded to 5×10^2 CFU in the sample. Bacterial counts were subject to a log transformation as follows: log (count +1). All statistical tests were 2-tailed and conducted at a significance level of $p \leq 0.05$.

The protocol and the informed consent were approved by the local human subjects review committee.

Results

Table 1 summarizes the demographic characteristics and smoking history of the three treatment groups. A total of 28 females and 19 males were randomly assigned to one of the three treatment modalities according to a computer-generated code. No statistically significant differences were found between any of the treatment groups.

The principal parameters evaluated were mean changes in Pocket Probing Depth (PPD) and Probing Attachment Level (PAL) as well as changes in total aerobic and anaerobic bacterial counts (i.e. CFU/mL) and specific periodontal pathogens between baseline and Week 18.

Figure 1 shows for each subject a comparison of the ETSA total cultivable counts (TC) at baseline and 2 weeks later. The subjects are sorted first by treatment group and then by the baseline TCs. TC differences between groups were not significant at baseline. However, only 14 out of the 32 sites treated with Elyzol showed lower total counts after treatment than at baseline, whereas 20 out of the 30 sites treated with PerioChip and 24 out of 32 sites treated with Atridox showed lower counts. An increase or no change in TCs was observed in 18 sites treated with Elyzol ®, in 10 sites treated with PerioChip and in eight sites treated with Atridox. These differences were statistically significantly different by Chi-square analysis ($p < 0.02$). Figure 1 also indicates that the treatments seemed to be more effective in sites with baseline TCs exceeding 10^6 CFU/mL than in sites with lower counts. All nine sites in the Atridox group with a baseline TC $> 10^6$ CFU/mL showed a decrease to counts below 10^6. In the PerioChip group, this was achieved in eight out of 11 sites, and in the Elyzol gel group in

only four out of 10 sites. An analysis of variance on the TC change from baseline to Month 4, including the baseline TCs as a covariable did not show a statistically significant difference between the treatment groups.

Table 2 shows the frequency of detection of the seven target organisms monitored in this study. *Prevotella intermedia* and *Fusobacterium* spp. were the most frequently detected target organisms before as well after treatment. None of the three tested procedures was able to suppress these organisms significantly below detection levels. With regard to *Porphyromonas gingivalis*, at Week 2 all monitored sites in the Atridox group were negative, whereas one site was found positive in the PerioChip group, and three in the Elyzol group. *Actinobacillus actinomycetemcomitans* could no longer be detected at Week 2 in all but one site in the Atridox group, and in all but three in the two other groups. At Week 18, reductions were no longer significant and no significant difference between the three treatments existed.

Mean PAL changes for all experimental sites

Mean PAL changes for all subjects are presented in Table 3. All treatment groups showed a gain in PAL from baseline to Week 18. However, the within-group comparison revealed that only subjects treated with Atridox reached a statistically significant gain in mean PAL ($p = 0.001$). Between baseline and the Week 18 examinations, the patients treated with Atridox showed a significantly greater gain in mean PAL of 0.33 mm + 0.09 (SD) than the patients treated with Elyzol [0.03 mm + 0.09 (SD)] ($p = 0.03$). However, the gain in PAL of 0.16 mm + 0.10 (SD) found after the application of PerioChip was not significantly different from that obtained following the application of Atridox ($p = 0.27$).

Table 3. Attachment level changes at all experimental sites (mean ± SD)

Treatment group (n = subjects)	Baseline	Month 4	Change within group	Change vs. Atridox™
Elyzol ($n = 16$)	5.01 ± 0.35	4.98 ± 0.34	0.03 ± 0.10	$P = 0.014$
PerioChip ($n = 15$)	5.25 ± 0.46	5.09 ± 0.42	0.16 ± 0.12	$P = 0.078$
Atridox ($n = 16$)	4.61 ± 0.26	4.28 ± 0.23	0.33 ± 0.08[a]	

[a] $p = 0.001$

Table 4. Frequency distribution of attachment level changes at all experimental sites

	Elyzol®		PerioChip®		Atridox™	
(n = sites)	n	%	n	%	n	%
Gain ≥ 1 mm	57	34%	52	36%	71	42%
Gain ≥ 2 mm	13	8%	9	6%	15	9%
Loss ≥ 1 mm	48	29%	31	22%	29	17%[a]
Loss ≥ 2 mm	11	7%	6	4%	4	2%

[a] $p = 0.013$ vs. Elyzol.

Table 5. Probing depth changes at all experimental sites (mean ± SD)

Treatment group (n = subjects)	Baseline	Month 4	Change within group	Change vs. Atridox™
Elyzol ($n = 16$)	5.38 ± 0.07	5.12 ± 0.12	0.25 ± 0.10[a]	$p = 0.42$
PerioChip ($n = 15$)	5.31 ± 0.14	5.04 ± 0.15	0.27 ± 0.10[b]	$p = 0.53$
Atridox ($n = 16$)	5.22 ± 0.09	4.90 ± 0.09	0.33 ± 0.08[a]	

[a] $p = 0.018$.
[b] $p = 0.021$.
[c] $p = 0.001$.

Frequency distribution of PAL changes for all experimental sites

Table 4 presents the percentage of PAL changes for all sites between baseline and Week 18. The proportion of sites that exhibited a gain of ≥ 1 mm or a gain of ≥ 2 mm PAL was similar for all three treatment groups. However, the group treated with Elyzol showed a significantly higher proportion of sites that lost ≥ 1 mm PAL when compared to the group treated with Atridox (29 vs. 17% at $p = 0.013$).

Mean PPD changes for all experimental sites

Mean changes in PPD for all subjects are presented in Table 5. All treatment groups showed a significant decrease in PPD from baseline to Week 18. The decrease in PPD was 0.25 mm ± 0.10 (SD) in the group treated with Elyzol ($p = 0.018$), 0.27 mm ± 0.10 (SD) in the group treated with PerioChip ($p = 0.021$) and 0.33 mm ± 0.08 (SD) in the group treated with Atridox ($p = 0.001$). However, no significant differences were found when PPD reduction in the patients treated with Atridox was compared with those treated with Elyzol or PerioChip.

Frequency distribution of PPD changes at all experimental sites

Table 6 shows the frequency of PPD changes for all sites treated. No significant differences in the proportion of sites showing a decrease or an increase in probing depth of ≥ 1 mm or ≥ 2 mm were found when the three treatment groups were compared.

Mean PAL changes in sites with 5 mm PPD at baseline

Table 7 presents PAL changes for sites with 5 mm PPD at baseline. Sites treated with either PerioChip or Atridox showed a gain in PAL between baseline and Week 18, whereas sites treated with Elyzol showed a minimal loss in PAL. However, only the PAL gain observed in the sites treated with Atridox reached statistical significance ($p = 0.001$). The gain in PAL of 0.23 mm + 0.07 (SD) found after the application of Atridox was significantly different from that obtained following the application of Elyzol ($p = 0.0007$), but only approached statistical significance

Table 6. Frequency distribution of probing depth changes at all experimental sites

	Elyzol®		PerioChip®		Atridox™	
(n = sites)	n	%	n	%	n	%
Decrease ≥1 mm	59	34%	42	29%	59	35%
Decrease ≥2 mm	18	10%	8	6%	8	5%
Increase ≥1 mm	25	15%	17	12%	15	9%
Increase ≥2 mm	5	3%	1	1%	0	0%

Table 7. Attachment level changes at sites with 5 mm probing depth at baseline (mean ± SD)

Treatment group (n = sites)	Baseline	Month 4	Change within group	Change vs. Atridox™
Elyzol (n = 128)	4.57 ± 0.12	4.63 ± 0.13	− 0.06 ± 0.08	p = 0.0007
PerioChip (n = 119)	4.48 ± 0.14	4.40 ± 0.14	0.08 ± 0.08	p = 0.056
Atridox (n = 144)	4.34 ± 0.11	4.09 ± 0.10	0.23 ± 0.07[a]	

[a] p = 0.001.

Table 8. Frequency distribution of attachment level changes at sites with 5 mm probing depth at baseline

	Elyzol®		PerioChip®		Atridox™	
(n = sites)	n	%	n	%	n	%
Gain ≥1 mm	38	31%	41	35%	58	41%
Gain ≥2 mm	5	4%	8	7%	11	8%
Loss ≥1 mm	37	30%	28	24%	24	17%[a]
Loss ≥2 mm	10	8%	5	4%	4	3%

[a] p = 0.012 vs. Elyzol ®

Table 9. Probing depth changes at sites with 5 mm probing depth at baseline (mean ± SD)

Treatment group (n = sites)	Baseline	Month 4	Change within group	Change vs. Atridox™
Elyzol (n = 128)	5.00 ± 0.00	4.80 ± 0.09	0.20 ± 0.09[a]	p = 0.599
PerioChip (n = 119)	5.00 ± 0.00	4.82 ± 0.06	0.18 ± 0.06[b]	p = 0.383
Atridox (n = 144)	5.00 ± 0.00	4.75 ± 0.06	0.25 ± 0.06[c]	

[a] p = 0.025.
[b] p = 0.006.
[c] p < 0.001.

when compared to that of the sites treated with PerioChip (p = 0.056).

Frequency distribution of PAL changes in sites with 5 mm PPD at baseline

Table 8 presents the percentage of PAL changes for all sites between baseline and Week 18. No significant differences were observed in the proportion of sites gaining ≥1 mm or ≥2 mm when the three treatment groups were compared. However, the percentage of sites treated with Atridox that lost ≥1 mm was significantly lower when compared to that of the sites treated with Elyzol (17 vs. 30% at p = 0.012).

Mean PPD changes in sites with 5 mm PPD at baseline

Table 9 presents PPD changes for sites with 5 mm PPD at baseline. All treatment groups showed a statistically significant decrease in PPD from baseline to Week 18. The decrease in PPD amounted to 0.20 mm ± 0.09 (SD) in the sites treated with Elyzol (p = 0.025), to 0.18 mm ± 0.06 (SD) in the sites treated with PerioChip (p = 0.006) and to 0.25 mm ± 0.06 (SD) in the sites treated with Atridox (p < 0.001). However, no significant differences were found when the PPD reduction in the sites treated with Atridox was compared with that of the sites treated with either Elyzol or PerioChip.

Frequency distribution of PPD changes in sites with 5 mm PPD at baseline

Table 10 presents the percentage of PPD changes for sites with 5 mm PPD at baseline to Week 18. The proportion of sites treated with PerioChip that decreased by ≥1 mm in depth (12%) was significantly lower when compared to those treated with Elyzol ®(32%) or Atridox (31%) (p < 0.001). Furthermore, the percentage of sites treated with Atridox that increased by ≥1 mm in depth (9%) was significantly lower when compared to that of sites treated with PerioChip (25%) (p < 0.001). No sites treated with Atridox showed an increase in depth of ≥2 mm. This was statistically significant when compared to the sites treated with either Elyzol (3%) or PerioChip (4%) (p = 0.047 and p = 0.018 respectively).

Mean PAL changes in sites with 6 mm PPD at baseline

Table 11 presents PAL changes for sites with 6 mm PPD at baseline. All treatment groups showed gain in PAL from baseline to Week 18. Sites treated with either Elyzol or Atridox showed a significant gain in PAL of 0.44 mm ± 0.19 (SD) and 0.59 mm ± 0.22 (SD) (p = 0.023 and p = 0.014, respectively). However, no statistically significant differences were found when the PAL gain in the sites treated with Atridox was compared with that of the sites treated with either Elyzol or PerioChip.

Frequency distribution of PAL changes in sites with ≥6 mm PPD at baseline

Table 12 presents the frequency distribution of PAL changes for sites ≥6 mm at baseline to Week 18. No significant differences in the proportion of sites gaining or losing ≥1 mm and ≥2 mm PAL were found when the three treatment groups were compared.

Mean PPD changes in sites with ≥6 mm PPD at baseline

Table 13 presents PPD changes for sites with PPD ≥6 mm at baseline. All three treatment groups showed a statistically significant decrease in PPD from baseline to Week 18. The decrease in PPD was 0.56 mm ± 0.17 (SD) in the group treated with Elyzol ($p = 0.003$), 0.46 mm ± 0.20 (SD) in the group treated with PerioChip ($p = 0.031$) and 0.70 mm ± 0.20 (SD) in the group treated with Atridox ($p = 0.002$). However, no statistically significant differences were found when PPD reduction in the sites treated with Atridox was compared with that of the sites treated with either Elyzol or PerioChip.

Table 10. Frequency distribution of probing depth changes at sites with 5 mm probing depth at baseline

	Elyzol®		PerioChip®		Atridox™	
(*n* = sites)	*n*	%	*n*	%	*n*	%
Decrease ≥1 mm	40	32%[a]	14	12%	44	31%[b]
Decrease ≥2 mm	10	8%[c]	0	0%	4	3%
Increase ≥1 mm	21	17%	30	25%	13	9%[d]
Increase ≥2 mm	4	3%[e]	5	4%[f]	0	0%

[a] $p = 0.001$ vs. PerioChip.
[b] $p < 0.001$ vs. PerioChip.
[c] $p = 0.002$ vs. PerioChip.
[d] $p < 0.001$ vs. PerioChip.
[e] $p = 0.047$ vs. Atridox.
[f] $p = 0.018$ vs. Atridox.

Table 11. Attachment level changes at sites with ≥6 mm probing depth at baseline (mean ± SD)

Treatment group (*n* = sites)	Baseline	Month 4	Change within group	Change vs. Atridox™
Elyzol ($n = 48$)	6.56 ± 0.22	6.02 ± 0.25	0.44 ± 0.19[a]	$p = 0.336$
PerioChip ($n = 24$)	7.56 ± 0.46	7.21 ± 0.45	0.35 ± 0.18	$p = 0.117$
Atridox ($n = 27$)	6.89 ± 0.37	6.30 ± 0.36	0.59 ± 0.22[b]	

[a] $p = 0.023$.
[b] $p = 0.014$.

Table 12. Frequency distribution of attachment level changes at sites = 6 mm probing depth at baseline

	Elyzol®		PerioChip®		Atridox™	
(*n* = sites)	*n*	%	*n*	%	*n*	%
Gain = 1 mm	19	44%	11	46%	13	48%
Gain = 2 mm	8	19%	1	4%	4	15%
Loss = 1 mm	10	23%	3	13%	5	19%
Loss = 2 mm	1	2%	1	4%	0	0%

Table 13. Probing depth changes at sites with ≥6 mm probing depth at baseline (mean ± SD)

Treatment group (*n* = sites)	Baseline	Month 4	Change within group	Change vs. Atridox™
Elyzol ($n = 48$)	6.52 ± 0.09	5.96 ± 0.20	0.56 ± 0.17[a]	$p = 0.578$
PerioChip ($n = 24$)	6.75 ± 0.19	6.29 ± 0.22	0.46 ± 0.20[b]	$p = 0.282$
Atridox ($n = 27$)	6.56 ± 0.21	5.85 ± 0.25	0.70 ± 0.20[c]	

[a] $p = 0.003$.
[b] $p = 0.031$.
[c] $p = 0.002$.

Frequency distribution of PPD changes in sites with ≥6 mm PPD at baseline

Table 14 presents the proportions of PPD changes for sites with PPD ≥6 mm between baseline and Week 18. The proportion of the sites treated with PerioChip that decreased by ≥1 mm in depth (13%) was significantly lower when compared to that of the sites treated with either Elyzol ®(41%) or Atridox (56%) ($p = 0.027$ and $p < 0.003$, respectively). Conversely, the percentage of the sites treated with PerioChip that increased by ≥1 mm in depth (48%) was significantly higher when compared to that of the sites treated with either Elyzol (9%) or Atridox (7%) ($p < 0.001$ and $p = 0.003$, respectively).

Discussion

This prospective RCT was designed to compare a possible adjunctive effect of local drug delivery devices applied into residual periodontal pockets defined as ≥5 mm following initial periodontal therapy. It has to be understood that prior to this application, the mechanical debridement performed during initial therapy in conjunction with improved oral hygiene standards had a major effect on clinical treatment outcomes as well as on microbiological colonization. Generally, a PPD reduction of approximately 1–1.5 mm in medium size pockets of 4–6 mm baseline PPD and of 2–2.5 mm PPD reduction in deeper sites exceeding 6 mm baseline PPD has to be expected (Badersten et al. 1981, 1984, Hill et al. 1981, Morrison et al. 1980, Pihlstrom et al. 1983, Westfelt et al. 1985). This occurs concomitantly with a PAL gain of approximately 0.5 mm and 1.5 mm in the medium size (4–6 mm PPD) and the deep (≥6 mm PPD) pockets, respectively. Any additional pocket reduction or gain of PAL would therefore represent a true clinical benefit and hence may decrease the need for additional periodontal surgery. Mechanical debridement during initial periodontal treatment results in significant changes in the composition of the subgingival microbiota and generally reduces the bacterial load by at least 2 logs (Loos et al. 1988, Magnusson et al. 1984, Mousquès et al. 1980, Pedrazzoli et al. 1992, Sbordone et al. 1990). The altered

Table 14. Frequency distribution of probing depth changes at sites with ≥ 6 mm probing depth at baseline

	Elyzol®		PerioChip®		Atridox™	
(*n* = sites)	*n*	%	*n*	%	*n*	%
Decrease ≥ 1 mm	19	41%[a]	3	13%	15	56%[b]
Decrease ≥ 2 mm	8	17%	1	4%	4	15%
Increase ≥ 1 mm	4	9%[c]	11	48%	2	7%[d]
Increase ≥ 2 mm	1	2%	2	9%	0	0%

[a] $p = 0.027$ vs. PerioChip.
[b] $p = 0.003$ vs. PerioChip.
[c] $p < 0.001$ vs. PerioChip.
[d] $p = 0.003$ vs. PerioChip.

bacterial composition following mechanical debridement represents a basis for the healing of the periodontal tissues finally being expressed as pocket reduction and gain of PAL.

In the present study, the microbiological parameters did not indicate a dramatic decrease in the quantity and composition of the subgingival microbiota due to any of the three treatments rendered. Furthermore, none of the treatments were able to eliminate the presence of putative periodontal pathogens permanently. One explanation may be the re-establishment of a subgingival biofilm since initial therapy. This phenomenon has been shown to protect microorganisms from antimicrobial agents. In the present study, subjects had received full-mouth scaling and root planing 2–4 months prior to baseline examination but were not mechanically re-treated before the antimicrobial agents were placed. In other studies (Kinane & Radvar 1999, Radvar et al. 1996), patients with persisting pockets were treated by scaling and root planing before the agents were placed. Tetracycline fibers, minocycline gel and metronidazole gel all seem to offer some benefit over scaling and root planing alone. While it was evident from the analysis of the detection frequency of putative periodontal pathogens that those were not eliminated by any of the three locally applied antimicrobials, an immediate effect of PerioChip and Atridox on the total cultivable microbiota (TCs) may be suggested. Between baseline and 2 weeks later, of the 30 sites treated with PerioChip, 20 showed lower and nine higher TCs. Following treatment with Atridox, 24 out of 32 sites showed lower and only seven higher TCs. Treatment with Elyzol appeared to have no microbiological effects whatsoever; only 14 out of the 32 sites showed lower TCs, while 13 sites yielded higher TCs and five sites remained unchanged. The statistical analysis revealed significant differences longitudinally among treatments, suggesting a higher potential for the reduction of bacteria after application of both PerioChip and Atridox. The microbiological outcomes of the present study may at least in part explain the relatively small beneficial clinical effects as well as the differences obtained with the various local adjunctive therapies.

All the adjunctive local antimicrobial therapies showed beneficial clinical outcomes, with an average PPD reduction of approximately 0.3 mm concomitant with a gain in PAL varying between 0.03 and 0.33 mm. As mentioned, these clinical benefits are in addition to the already marked benefits obtained by the initial periodontal mechanical debridement and have to be considered clinically relevant, although on a very modest level. It was also clear from the analysis of the mean values of clinical outcomes that application of Atridox yielded significantly higher benefits over both other therapies in terms of PPDs and PALs. On the other hand, the mean PAL of the sites treated with Elyzol and PerioChip did not reveal any longitudinal changes, indicating that the value of their application adjunctive to mechanical debridement has to be questioned.

Analyzing the frequency distribution of single sites being treated with one of three local antimicrobial adjunctive drugs, it is again evident that Atridox resulted in the highest proportion of clinical benefits as expressed by decrease in PPD and increase in PAL. Also, it is clear that adjunctive Atridox application yielded the fewest deteriorating sites as expressed by increasing PPD (9%) and loss of PAL (19%). On the other hand, therapy with Elyzol after initial mechanical debridement resulted in twice as many sites with increasing PPD (18%) and further loss of PAL (36%). It has to be remembered, however, that only residual pockets of ≥ 5 mm PPD following mechanical debridement were included in the present RCT.

Analyzing the deep lesions with a PPD of ≥ 6 mm following mechanical debridement, a significant clinical benefit was observed for all three treatment modalities. PPD reductions and PAL gain were twice those of the mean scores of all sites included in the study. However, owing to the small number of these deep sites, varying between 24 and 48 for the various therapies, no statistically significant differences were observed among the groups. Nevertheless, Atridox application resulted in a clearly superior clinical outcome, with a PPD reduction of 0.7 mm and a PAL increase of 0.6 mm following mechanical debridement.

Again, analyzing the frequency distribution of the deep pockets with PPD ≥ 6 mm, the greatest clinical benefits were observed for adjunctive Atridox therapy with 71% of the sites yielding decreased PPD and 63% of the sites with increasing PAL, as opposed to only 17% of the sites with decreased PPD and 50% of the sites with increasing PAL following application of PerioChip. In these deep pockets, Elyzol therapy also resulted in significantly more sites with decreased PPD than did PerioChip application. It has to be anticipated that very deep lesions of PPD ≥ 6 mm may be reinfected within the course of 2 months and eventually show further progression. In the present RCT, 11% of the Elyzol -treated sites and even 57% of the PerioChip-treated lesions yielded increased PPD as opposed to only 7% following Atridox application.

From a clinical point of view, the microbiological changes following the application of Elyzol were negligible with an equal number of sites having slightly reduced or slightly increased total bacterial counts. Also, no changes in PAL were seen on an average in residual pockets ≥ 5 mm following Elyzol treatment. The value of local application of Elyzol adjunctive to mechanical debridement has therefore to be questioned, even when – according to the manufacturer's recommendations – two applications of the antibiotic were done. This is most likely due to the very

low half-time efficacy of the drug, as demonstrated in *in vivo* studies (Stoltze 1992, 1995).

While the application of PerioChip adjunctive to mechanical therapy resulted in clinical benefits of moderate magnitude between those obtained with Elyzol and those with Atridox, PerioChip application had very poor treatment outcomes in deeper periodontal lesions of 6 mm. A remarkably higher proportion of those sites (57%) yielded increased PPD 4 months after the application of PerioChip. This may indicate that the dimensions of the PerioChip wafer do not allow efficacy beyond a certain PPD. Since PerioChip application was moderately effective in inducing PPD reduction and gain in PAL in all periodontal sites studied, it may be concluded that the application of PerioChip should not be recommended for residual pockets of ≥ 6 mm.

The application of Atridox into residual pockets of ≥ 5 mm adjunctive to mechanical debridement resulted in clinical outcomes superior to those of the two other local antimicrobial agents tested. This was concomitant with superior proportions of sites with reduced total bacterial counts, indicating that the additional successful suppression of the subgingival microbiota following mechanical debridement may indeed lead to further shrinkage in residual pockets and tightening of the periodontal tissues, resulting in clinical attachment gains. However, the order of magnitude to be expected from such an effort may be considered moderate in comparison with the outcomes of initial periodontal therapy.

In conclusion, this RCT has demonstrated that clinical outcomes were almost three times as high in deeper lesions of ≥ 6 mm as compared to residual pockets of 5 mm. From a clinical point of view, Atridox application therefore may be recommended as adjunctive to mechanical debridement in residual periodontal lesions of at least 6 mm. It has to be kept in mind, however, that the clinical improvements expected with such a treatment approach may not eliminate the need for additional surgical therapy.

Acknowledgments

This study was partially supported by the Clinical Research Foundation (CRF) for the Promotion of Oral Health of the University of Berne, Switzerland, and Atrix Laboratories Inc., Fort Collins, Colorado, USA. The authors would like to thank Graham Carron and Barbara Pors for statistical assistance. The competent laboratory assistance of Mrs. Antonia Colangelo, Regula Hirschi and Marianne Weibel is highly appreciated.

Zusammenfassung

Lokale antimikrobielle Therapie nach initialer Parodontalbehandlung

Ziel: Das Ziel dieser einfachblinden, randomisierten, klinischen Studie mit Parallel-Design (RCT) war es, den klinischen und mikrobiologischen Effekt von 3 Medikamententräger aus biologisch abbaubaren Polymeren, welche nach der initialen Parodontalbehandlung in parodontale Taschen eingebracht werden, zu evaluieren.

Methoden: Siebenundvierzig Patienten (28 Frauen und 19 Männer) mit einem Durchschnittsalter von 51 Jahren (Alterspanne 29-71) wurden bei Aufnahme in die Studie (Woche 0) und nach 18 Wochen einer parodontalen Untersuchung unterzogen. Die beinhaltete die Messung des Plaque-Index (PlI), der Sondierungsblutung, der klinischen Sondierungstiefe (PPD) und des klinischen Attachmentniveaus an 6 Stellen pro Zahn. Zwei bis 4 Monate vor der Eingangsuntersuchung erhielten alle Patienten eine initiale Parodontalbehandlung, welche Motivation, Instruktion von Mundhygienemaßnahmen sowie scaling und Wurzelglättung aller Zähne einschloss. In der Behandlungssitzung (z.B. Woche 2) wurde nach einem Zufallsprinzip bestimmt, ob die Patienten Atridox™, Elyzol® Dental Gel oder PerioChip® an allen verbliebenen Taschen mit Sondierungstiefe ≥ 5 mm bei gleichzeitiger positiver Sondierungsblutung erhalten sollten. In Übereinstimmung mit den Empfehlungen wurde Elyzol Dental Gel ein zweites Mal 7 Tage später appliziert. Zusätzlich zur klinischen Evaluation wurden vor der Behandlung (Woche 2) und in Woche 4 und 18 subgingivale mikrobiologische Proben entnommen. Die Varianz/Kovarianz-Analyse wurde verwendet, um die Veränderung der klinischen Parameter zwischen der Eingangsuntersuchung und der Woche 18 zu evaluieren.

Ergebnisse: Zwischen der Eingangs- und der Woche 18-Untersuchung zeigten die Patienten, die mit Atridox behandelt wurden einen signifikant höheren durchschnittlichen Attachmentgewinn von 0.33 ± 0.09 (SD) als Patienten die mit Elyzol Dental Gel behandelt wurden [0.03 mm ± 0.09 (SD)] (p = 0.03). Jedoch unterschied sich der Attachmentgewinn von 0.16 mm ± 0.10 (SD) der für PerioChip vorgefunden wurde nicht signifikant von dem, den man nach der Behandlung mit Atridox erzielt hatte (p = 0.27). Im Gegensatz zu den Taschen, die mit Elyzol Dental Gel behandelt wurden und von denen 34% ≥ 1 mm und 8% ≥ 2 mm Attachmentgewinn aufwiesen zeigten Taschen, die mit Atridox behandelt wurden bei 42% ≥ 1 mm und bei 9% ≥ 2 mm Attachmentgewinn. Von den Taschen, die nach abgeschlossener initialer Parodontalbehandlung mit PerioChip behandelt wurden hatten 36% ≥ 1 mm und 6% ≥ 2 mm Attachmentgewinn.

Schlussfolgerungen: Die Applikation von 3 biologisch abbaubaren Medikamententrägern, die nach initialer Parodontalbehandlung erprobt wurden, hatte für Atridox einen statistisch signifikant Attachmentgewinn zum Ergebnis und führte während der Studienperiode für alle 3 Träger zu einer signifikanten Reduktion der PPD. Desweiteren zeigte sich, dass, wenn man Taschen, die mit Atridox behandelt wurden, mit Taschen, die mit Elyzol behandelt wurden, verglich, ein signifikanter Unterschied im Attachmentgewinn (0.3 mm) beobachtet wurde.

Résumé

Traitement local antimicrobien après traitement initial parodontal. Une étude clinique et randomisée comparant 3 polymères biodégradables à libération maintenue.

But: Le but de cette étude clinique en simple aveugle, randomisée, parallèle (RCT) était d'évaluer les effets cliniques et microbiologiques de trois polymères biodégradables, à liberation maintenue mis en place dans des poches parodontales après un traitement initial.

Méthodes: 47 patients (28 femmes et 19 hommes) d'un,ge moyen de 51 ans (de 29 à 71 ans) subirent un examen parodontal initial (c'est à dire lors de la semaine 0) et un autre après 18 semaines. Cet examen comprenait la mise en évidence de l'indice de plaque (PlI), le saignement au sondage, (BOP), les profondeurs de poche déterminées par sondage (PPD), et les niveaux d'attache (PAL) sur 6 sites par dents. 2 à 4 mois avant le début de l'étude, tous les sujets ont reçu une thérapeutique initiale, avec motivation, instruction d'hygiène orale et détartrage et surfaçage radiculaire complet. Lors du rendez vous thérapeutique (Semaine 2), les patients étaient répartis au hasard pour recevoir Atridox™, Elyzol® Dental Gel or PerioChip® sur toutes les poches résiduelles présentant encore une profondeur de poche au sondage ≥ 5 mm et un BOP correspondant. Suivant les recommandations du fabricant, Elyzol Dental Gel fut appliqué une seconde fois, une semaine plus tard .En plus de l'évaluation clinique, des échantillons microbiologiques sous gingivaux furent prélevés avant le traitement (semaine 2) et aux semaines 4 et 18. L'analyse de la variance et de la covariance ont été utilisées pour évaluer les modifications entre le début de l'étude et la semaine 18 pour les paramètres cliniques.

Résultats: Entre l'examen initial et l'examen final, les sujets traités par Atridox présentaient un gain d'attache moyen significativement plus important de 0.33 mm ± 0.09 (SD) que les sujets traités par Elyzol Dental Gel [0.03 mm ± 0.09 (SD)] (p = 0.03). Cependant, le gain obtenu après l'application de PerioChip (0.16 mm ± 0.10) (SD) ne différait

pas significativement de celui obtenu après utilization de Atridox ($p = 0.27$). Parmi les sites traité par Atridox , 42% gagnaient ≥ 1 mm de PAL et 9% ≥ 2 mm de PAL. Au contraire, des sites traités par Elyzol Dental Gel, parmi lesquels, 34% gaganient ≥ 1 mm de PAL et 8% ≥ 2 mm de PAL. Parmi les sites traités avec PerioChip, 36% gagnaient ≥ 1 mm et 6% ≥ 2 mm de PAL après l'execution d'un traitement initial.

Conclusions: L'application des trois systèmes biodégradables à libération maintenue, essayés après execution d'un traitement parodontal initial a entraîné un gain d'attache moyen statistiquement significatif pour Atridox et une réduction significative des profondeurs de poche pour les 3 systèmes pendant la période d'étude. De plus, lorsque les sites traités par Atridox étaient comparés aux sites traités par Elysol, une différence de gain d'attache significative (0.3 mm) était observée.

References

Ainamo, J., Lie, T., Ellingsen, B. H., Hansen, B. F., Johansson, L. A., Karring, T., Kisch, J. , Paunio, K. & Stoltae, K.(1992) Clinical responses to subgingival application of a metronidazole 25% gel compared to the effect of subgingival scaling in adult periodontitis. *Journal of Clinical Periodontology* **19**, 723–729.

Badersten, A., Nilvéus, R. & Egelberg, J. (1981) Effect of nonsurgical periodontal therapy. I. Moderately advanced periodontitis. *Journal of Clinical Periodontology* **8**, 57–72.

Badersten, A., Nilvéus, R. & Egelberg, J. (1984) Effect of nonsurgical periodontal therapy. II. Severely advanced periodontitis. *Journal of Clinical Periodontology* **11**, 63–76.

Garrett, S., Adams, D. F., Bogle, G., Donly, K., Drisko, C. H., Hallmon, W. W., Hancock, E. B., Hanes, P., Hawley, C. E., Johnson, L., Kiger, R., Killoy, W., Mellonig, J. T., Raab, F. J., Ryder, M., Stoller, N., Polson, A., Wang, H. L., Wolinsky, L. E., Yukna, R. A., Harrold, C. Q., Hill, M., Johnson, V. B. & Southard, G. L., (2000) The effect of locally delivered controlled-release doxycycline or scaling and root planing on periodontal maintenance patients over 9 months. *Journal of Periodontology* **71**, 22–30.

Garrett, S., Johnson, L., Drisko, C. H., Adams, D. F., Bandt, C. L., Beiswanger, B., Bogle, G. (1999) Two multi-center studies evaluating locally delivered doxycycline hyclate, placebo control, oral hygiene and scaling and root planing in the treatment of periodontitis. *Journal of Periodontology* **70**, 490–503.

Greenstein, G. & Polson, A. (1998) The role of local drug delivery in the management of periodontal diseases: a comprehensive review. *Journal of Periodontology* **69**, 507–520.

Hill, R. W., Ramfjord, S. P., Morrison, E. C., Appleberry, E. A., Caffesse, R. G., Kerry, G. J. & Nissle, R. R. (1981) Four types of periodontal treatment compared over two years. *Journal of Periodontology* **52**, 655–662.

Jeffcoat, M. K., Bray, K. S., Ciancio, S. G., Dentino, A. R., Fine, D. H., Gordon, J. M., Gunsolley, J. C., Killoy, W. J., Lowenguth, R. A., Magnusson, N. I., Offenbacher, S., Palcanis, K. G., Proskin, H. M., Finkelman, R. D. & Flashner, M. (1998) Adjunctive use of a subgingival controlled-release chlorhexidine chip reduces probing depth and improves attachment level compared with scaling and root planing alone. *Journal of Periodontology* **69**, 989–997.

Keller, W., Brägger, U. & Mombelli, A. (1998) Peri-implant microflora of implants with cemented and screw retained suprastructures. *Clinical Oral Implants Research* **9**, 209–217.

Kinane, D. F. & Radvar, M. (1999) A six-month comparison of three periodontal local antimicrobial therapies in persistent periodontal pockets. *Journal of Periodontology* **70**, 1–7.

Klinge, B., Attström, R., Karring, T., Kisch, J., Lewin, B. & Stoltze, K. (1992) Three regimens of topical metronidazole compared with subgingival scaling on periodontal pathology in adults. *Journal of Clinical Periodontology* **19**, 708–714.

Lie, T., Bruun, G. & Boe, O. E. (1998) Effects of topical metronidazole and tetracycline in treatment of adult periodontitis. *Journal of Periodontology* **69**, 819–827.

Loos, B., Claffey, N. & Crigger, M. (1988) Effects of oral hygiene measures on clinical and microbiological prameters of periodontal disease. *Journal of Clinical Periodontology* **15**, 211–216.

Magnusson, I., Lindhe, J., Yoneyama, T. & Liljenberg, B. (1984) Recolonization of a subgingival microbiota following scaling in deep pockets. *Journal of Clinical Periodontology* **11**, 193–207.

Morrison, E. C., Ramfjord, S. P. & Hill, R. W. (1980) Short-term effects of initial, nonsurgical periodontal treatment (hygienic phase). *Journal of Clinical Periodontology* **7**, 199–211.

Mousquès, T., Listgarten, M. A. & Phillips, R. W. (1980) Effect of scaling and root planing on the composition of the human subgingival microbial flora. *Journal of Periodontal Research* **15**, 144–151.

Noyan, U., Yilmaz, S., Kuru, B., Kadir, T., Acar, O. & Buget, E. (1997) A clinical and microbiological evaluation of systemic and local metronidazole delivery in adult periodontitis patients. *Journal of Clinical Periodontology* **24**, 158–165.

Offenbacher, S. (1996) Periodontal diseases: pathogenesis. *Annals of Periodontology* **1**, 821–878.

Palcanis, K. G. (1996) Surgical pocket therapy. *Annals of Periodontology* **1**, 589–617.

Pedrazzoli, V., Kilian, M. & Karring, T. (1992) Comparative clinical and microbiological effects of topical subgingival application of metronidazole 25% dental gel and scaling in the treatment of adult periodontitis. *Journal of Clinical Periodontology* **19**, 715–722.

Pihlstrom, B. L., McHugh, R. B., Oliphant, T. H. & Ortiz-Campos, C. (1983) Comparison of surgical and nonsurgical treatment of periodontal disease. A review of current studies and additional results after 6, 1/2 years. *Journal of Clinical Periodontology* **10**, 524–541.

Polson, A. M., Garrett, S., Stoller, N. H., Bandt, C. L., Hanes, P. J., Killoy, W. J., Southard, G. L. , Duke, S. P., Bogle, G. C., Drisko, C. H. & Friesen, L. R. (1997) Multi-center comparative evaluation of subgingivally delivered sanguinarine and doxycycline in the treatment of periodontitis. II. Clinical results. *Journal of Periodontology* **68**, 119–126.

Radvar, M., Pourtaghi, N. & Kinane, D. F. (1996) Comparison of 3 periodontal local antibiotic therapies in persistent periodontal pockets. *Journal of Periodontology* **67**, 860–865.

Rams, T. E. & Slots, J. (1996) Local delivery of antimicrobial agents in the periodontal pocket. *Periodontology 2000* **10**, 139–159.

Riep, B., Purucker, P. & Bernimoulin, J. P. (1999) Repeated local metronidazole-therapy as adjunct to scaling and root planing in maintenance patients. *Journal of Clinical Periodontology* **26**, 710–715.

Rudhart, A., Purucker, P., Kage, A., Hopfenmüller, W. & Bernimoulin, J. P. (1998) Local metronidazole application in maintenance patients. Clinical and microbiological evaluation. *Journal of Periodontology* **69**, 1148–1154.

Sbordone, L., Ramaglia, L., Gulletta, E. & Iacono, V. (1990) Recolonization of the subgingival microflora after scaling and root planing in human periodontitis. *Journal of Periodontology* **61**, 579–584.

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* **22**, 121–135.

Slots, J. (1982) Selective medium for isolation of *Actinobacillus actinimycetemcomitans*. *Journal of Clinical Microbiology* **15**, 606–609.

Soskolne, W. A., Heasman, P. A., Stabholz, A., Smart, G. J., Palmer, M., Flashner, M. & Newman, H. N. (1997) Sustained local delivery of chlorhexidine in the treatment of periodontitis: a multi-center study. *Journal of Periodontology* **68**, 32–38.

Stelzel, M., Flores-de-Jacoby, L. (1996) Topical metronidazole application compared with subgingival scaling. A clinical and microbiological study on recall patients. *Journal of Clinical Periodontologyl* **23**, 24–29.

Stelzel, M., Flores-de & Jacoby, L. (1997) Topical metronidazole application in recall

patients. Long-term results. *Journal of Clinical Periodontology* **24**, 914–919.

Stoltze, K. (1992) Concentration of metronidazole in periodontal pockets after application of a metronidazole 25% dental gel. *Journal of Clinical Periodontology* **19**, 698–701.

Stoltze, K. (1995) Elimination of Elyzol 25% Dentalgel matrix from periodontal pockets. *Journal of Clinical Periodontology* **22**, 185–187.

Syed, S. A., Svanberg, M. & Svanberg, G. (1980) The predominant cultivable dental plaque flora of beagle dogs with gingivitis. *Journal of Periodontal Research* **15**, 123–136.

Westfelt, E., Bragd, L., Socransky, S. S., Haffajee, A. D., Nyman, S. & Lindhe, J. (1985) Improved periodontal conditions following therapy. *Journal of Clinical Periodontology* **12**, 283–293.

Zambon, J. J. (1996) Periodontal diseases: Microbial factors. *Annals of Periodontology* **1**, 879–925.

Address:
Dr Giovanni E. Salvi
University of Berne
School of Dental Medicine
Freiburgstrasse 7
CH-3010 Berne
Switzerland

Tel: +41 31 632 25 77
Fax: +41 31 632 49 15
e-mail: SALVI@zmk.unibe.ch