Efficacy and Safety of Adjunctive Local Moxifloxacin Delivery in the Treatment of Periodontitis

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Background: Moxifloxacin exerts excellent antibacterial activity against most putative periodontal pathogens and has been shown to kill bacteria in biofilm and host cells.

Methods: Patients with chronic periodontitis were randomly assigned to receive a single subgingival application of a 0.125%, 0.4%, or 1.25% moxifloxacin gel or placebo gel immediately after full-mouth scaling and root planing (SRP). Clinical efficacy measurements were assessed in sites with baseline probing depth (PD) of ≥5.4 mm at 6 weeks and 3 months and any adverse events were determined. In addition, putative periodontal pathogens and resistance of subgingival bacteria against moxifloxacin were assessed.

Results: Data of 57 patients were included in the statistical analysis. In all treatment groups, the PD decreased from baseline to 3 months, with the greatest reduction seen in patients treated with moxifloxacin 0.4% (1.5 ± 0.6 mm; $P = 0.023$ compared to placebo), followed by patients receiving moxifloxacin 1.25% (1.2 ± 0.4), moxifloxacin 0.125% (1.1 ± 1.1), and placebo (1.0 ± 0.6). No linear trend for PD reduction with increasing moxifloxacin concentrations was found. *Porphyromonas gingivalis* showed the greatest reduction in prevalence among the assessed pathogens, without any significant intergroup differences. No correlation or systematic relationship between adverse events, including bacterial resistance against moxifloxacin, and the investigational gels was found.

Conclusions: In periodontal pockets with PD of ≥5.4 mm, a single subgingival administration of a 0.4% moxifloxacin gel as an adjunct to SRP may result in additional PD reduction compared to SRP alone. In addition, the investigated moxifloxacin gels seem to be safe. *J Periodontol 2011;82:96-105.*

KEY WORDS

Anti-bacterial agents; biofilms; dental scaling; moxifloxacin; periodontal diseases; periodontitis.

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Moxifloxacin is a fourth-generation fluoroquinolone antibiotic with a broad antimicrobial activity against aerobic and anaerobic bacteria.[1,2] It exerts a bactericidal effect by specifically inhibiting adenosine triphosphate–dependent topoisomerase IV and topoisomerase II (DNA gyrase).[3] Moxifloxacin is used in the treatment of respiratory infections, including community-acquired pneumonia, acute bacterial exacerbation of chronic bronchitis,[4] and acute sinusitis, particularly in areas where drug-resistant *Streptococcus pneumonia* or *Haemophilus influenza* are common.[5] It also has been shown to be effective in the treatment of odontogenic abscesses,[6] multidrug-resistant viridians groups *Streptococcus* osteomyelitis of the mandible,[7] and complicated intra-abdominal infections.[8] Moxifloxacin is applied locally in the treatment of ophthalmic infections, such as keratitis and conjunctivitis,[9-11] and the prevention of bacterial infections after intraocular anterior segment surgery.[12]

Moxifloxacin exerts excellent antibacterial activity against a wide range of putative periodontal pathogens, including *Porphyromonas gingivalis*; *Tannerella forsythia* (previously *T. forsythensis*); *Prevotella* spp.; *Fusobacterium nucleatum*; *Actinomyces* spp.; *Peptostreptococcus* spp.; *Campylobacter rectus*;[13] and *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*).[14] Its bactericidal activity

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against biofilm-embedded *P. gingivalis*, *A. actinomycetemcomitans*, and *Streptococcus constellatus* was found to be superior to clindamycin, metronidazole, or doxycycline.[15] Moxifloxacin penetrates well into soft tissues[5] and is effective against intracellular periodontal pathogens.[16] When used in the treatment of periodontitis as an adjunct to scaling and root planing, systemic administration of moxifloxacin has provided superior outcomes compared to scaling and root planing in conjunction with systemic administration of doxycycline or scaling and root planing alone.[17]

MATERIALS AND METHODS

The study protocol, any amendments thereof, patient information, informed consent forms, and case record forms were reviewed and approved by the Ethics Committee of the Medical Chamber of Westphalia-Lippe. All study-related examinations were conducted in compliance with the Declaration of Helsinki (version of Washington 2002) and performed after the patient had been included into the study and written consent was obtained. The format of this report follows the recommendations put forth by the revised Consolidated Standards of Reporting Trials statement.[18]

Participants

Subjects aged 18 to 75 years presenting at the Clinic of Periodontology, Westphalian Wilhelm University, Münster, Germany, were screened from October 2004 to November 2005 for clinical signs consistent with the diagnosis of chronic periodontitis,[19] and for eligibility to participate in the clinical trial.

Patients meeting all of the following criteria were included in the study: 1) aged between 18 and 75 years; 2) ≥12 natural teeth present; 3) clinical and radiographic signs of moderate (clinical attachment level [CAL] of 3 to 4 mm) to severe (CAL of ≥5 mm) chronic periodontitis; 4) probing depth (PD) of ≥5.4 mm at ≥4 teeth without radiographic signs of apical periodontitis; 5) no subgingival debridement within the previous 12 months; and 6) willingness to comply with the study protocol.

Patients were excluded from the study if they met one or more of the following criteria: 1) existing systemic disease that may influence the severity or progression of periodontitis, in particular Down syndrome, HIV infection, or diabetes mellitus type 1 or type 2; 2) taking medications that may influence the periodontium (e.g., phenytoin, nifedipine, or non-steroidal anti-inflammatory drugs); 3) taking medication that may interact with moxifloxacin (e.g., antiarrhythmics, coumarin derivates, tricyclic antidepressants, antimalarials, or antihistamines); 4) existing tendon diseases or damage as a result of previous quinolone therapy; 5) cardiac arrhythmia; 6) liver diseases; 7) antibiotic premedication required for dental interventions; 8) systemic administration or local application of antibiotics within the previous 6 months; 9) concurrent or planned extensive dental or orthodontic treatments; 10) pregnancy or lactation; 11) intraoral piercing or other intraoral body jewelry; 12) unable or not willing to comply with the study protocol; 13) anticipated non-compliance with the examination and treatment appointments; and 14) systemic disease that may influence the severity or progression of periodontitis during the observation period of the study.

Interventions

Patients received scaling and root planing on 2 consecutive days ± 2 days in accordance with the one-stage full-mouth debridement protocol.[20] On each of these days scaling and root planing were performed in two quadrants under local anesthesia using sonic scalers with microtips.¶ To remove any residual biofilm or stain, supragingival and subgingival glycine powder# air polishing** was performed on all teeth. The therapeutic endpoint was defined as a clean root surface void of visible or clinically detectable remnants of biofilm or calculus. After debridement, a single dose of the investigational product was applied into all periodontal pockets with PD of ≥4 mm using a syringe with a blunt canula. The canula was inserted to the base of the periodontal pocket and gel was applied until excess gel flowed out of the pocket.

In the test groups, the investigational gel for local application contained moxifloxacin at concentrations of 0.125% (MOX 0.125), 0.4% (MOX 0.4), or 1.25% (MOX 1.25) and in the control group (control), a placebo gel was used. After treatment, patients were given thorough oral hygiene instructions and were asked to use an aminofluoride dentifrice†† and to refrain from using any mouthrinses during the course of the study. All interventional treatments were rendered by the same dental hygienist (DM) using two-fold loupe magnification. A study nurse (MG) dispensed all investigational drugs.

Objectives

This phase II clinical trial was performed to assess the efficacy and safety of a locally delivered moxifloxacin gel as an adjunct to scaling and root planing in the treatment of chronic periodontitis. In addition, the optimal moxifloxacin concentration of the investigational gel was to be determined.

Outcomes

Primary outcome measure. The primary outcome measure for efficacy was the change in PD between baseline and follow-up examinations at the sites of

¶ SONICflex with SONICflex paro tips #61 and #62, KaVo, Biberach an der Riss, Germany.
# Clinpro Prophypowder, 3M ESPE, Seefeld, Germany.
** EMS Airflow S1, EMS, Nyon, Switzerland.
†† Elmex, Gaba, Therwil, Switzerland.

four randomly selected investigational teeth in each patient showing PD of ≥5.4 mm.

Secondary outcome measures. As secondary efficacy outcome measures, changes in gingival recession (GR), CAL, bleeding on probing (BOP), suppuration, plaque index (PI),[21] and furcation invasion (FI) were assessed. PI was recorded before PD measurement and BOP was recorded immediately after probing. Furthermore, throughout the observation period, the prevalence of putative periodontal pathogens at the investigational sites was determined.

Efficacy measurements. Clinical measurements were assessed at baseline, 6, and 12 weeks on all teeth in patients included in the trial. Measurements included PD, CAL, GR, FI, PI, and BOP. All measurements with the exception of FI were taken with a computerized periodontal probe‡‡; PD, CAL, and GR measurements were recorded to the nearest 0.2 mm. For determining patient eligibility, PD readings of ≥6 mm as displayed on the computer screen, corresponding to PD measurements of ≥5.4, were used. PD, CAL, and BOP were measured at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) per tooth. FI was assessed using a furcation probe.§§

Subgingival plaque was collected from two randomly selected investigational teeth in each patient at sites that showed PD of ≥5.4 mm at baseline. The same sites were used for plaque sampling at 6 and 12 weeks. After removing supragingival plaque, subgingival plaque was collected using a sterile Gracey curet. Samples were placed in 200 μl of sterile distilled water and then placed in an ultrasonic bath‖‖ for 5 minutes at 37°C to disperse the plaque. Samples were vortexed and centrifuged at 12,000 × g for 1 minute to pellet the bacterial cells. DNA was isolated using a kit¶¶ according to the manufacturer's instructions. Samples from each site were analyzed separately for the presence of putative periodontal pathogens *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *Treponema denticola*, and *Streptococcus intermedius* using polymerase chain reaction as previously described.[22-25] Precautions as described by Kwok and Higuchi[26] were taken to prevent contamination.

Safety measurements. Adverse effects were assessed and documented throughout the duration of the study irrespective of any possible casual relationship with the study treatment. All adverse events were monitored until they adequately subsided and the outcome was documented in the case report forms. To assess the development of any bacterial resistance against moxifloxacin after treatment, subgingival plaque was collected in a randomly selected subpopulation of four patients from each treatment group from the two investigational teeth not used for assessment of putative periodontal pathogens. Supragingival plaque was gently removed and the teeth were air-dried and isolated with cotton rolls. One sterile endodontic paper point was inserted for 10 seconds in each site and placed in a transport tube containing 500 μl anaerobic Ringer-glycerin solution (12.5% glycerin in 0.25 concentrated Ringer solution). Plaque samples were taken at baseline, and weeks 1, 2, 4, 6, and 12, and processed within 15 minutes by sonication followed by vortexing for 10 seconds. Subsequently, the suspension was 20-fold serial diluted, streaked on non-selective blood agar (CDC agar) plates containing 5% defibrinated sheep blood supplemented with 5 mg/L hemin, and 1 mg/L vitamin K_1. Moreover the anaerobic blood agar was supplemented with 10 mg/L *N*-acetylmuramic acid for optimal cultivation of fastidious bacteria and incubated at 37°C for 7 days in an atmosphere of 85% N_2, 10% H_2, and 5% CO_2. Twelve isolates were randomly selected and resuspended in sterile Ringer solution, and streaked on blood agar plates. A moxifloxacin test## was placed on the plates and the plates incubated in anaerobic environment for 1 week. Strains resistant to moxifloxacin were identified using an appropriate system.*** Resistance was defined as resistance to the highest moxifloxacin concentration of the test strip. For moxifloxacin test-strips, the concentration gradient ranges from 0.002 to 32 μg/ml. Resistant bacteria showed no growth inhibition even at the highest moxifloxacin concentration of 32 μg/ml.

Methods to Enhance Quality of Measurements

By using a force-sensitive periodontal probe with direct data entry and double measurements for the primary outcome variable PD at the investigational sites, the quality and reliability of measurements were enhanced. All clinical examinations throughout the clinical trial were performed by the same licensed dentist (MZ). Calibration exercises were performed before the first patient was randomized and repeated every 2 months until the last patient exited the study. Calibration was determined by double measurements on 10 patients with moderate to severe periodontitis with a minimum of 12 teeth with a total of 1,440 sites. Agreement between double measurements was assessed by the variable agreement method described by Bland and Altman.[27] To ensure compliance with the study protocol, the study center was regularly monitored by an outside consultant (Hans-Jurgen Knopf, Bonn, Germany).

‡‡ Florida Probe, Gainesville, FL.
§§ Nabers Probe, Stoma, Emmingen-Liptingen, Germany.
‖‖ Bransonic 1510E, Branson, Dietzenbach, Germany.
¶¶ QIAamp DNA-Mini Kit, Quiagen, Hilden, Germany.
Etest, bioMerieux Deutschland, Nürtingen, Germany.
*** API system, bioMerieux Deutschland.

Sample Size

Using a one-sided type I error of $\alpha = 0.025$, a sample size of 15 patients in each treatment group ensured a power of 80% for a pairwise treatment group comparison with an effect size of 1.06 standard deviation units in an independent samples *t* test model. Because empirical data on the efficacy of the investigated moxifloxacin gel in the treatment of chronic periodontitis were lacking, an adaptive interim analysis was performed that included an option for sample size adjustment. After the interim analysis, the sample size was not changed and the study was not stopped earlier than initially planned.

Randomization

A permuted block randomization was generated by a consulting biostatistician (AV) to allocate patients to one of the four treatment groups. To ensure equal distribution of important risk factors and predictors for periodontitis, patients were stratified based on their smoking habit (non-smoker, <7 ppm carbon monoxide in exhaled air; smoker, ≥7 ppm carbon monoxide content in exhaled air) and disease extent (localized, <38% of teeth with PD of ≥6 mm; generalized, ≥38% of teeth with PD of ≥6 mm). Four teeth showing PPD of ≥5.4 mm at baseline were randomly selected as investigational teeth in each enrolled patient and used for primary outcome assessment. A subset of four patients in each treatment group was randomly selected to assess any changes in the susceptibility of subgingival bacteria against moxifloxacin after treatment.

Investigational drug and placebo gels were provided in labeled 10-ml vials. Allocation concealment was ensured by using packaging and labeling that did not reveal the content of the investigational drug vials and by using a central telephone service to assign investigational drug vial numbers to patients. At the treatment appointment, the study nurse dispensed the assigned investigational drug vial to the dental hygienist, who rendered all intervention treatments.

Masking

The trial was conducted in a double-masked, placebo-controlled manner. Neither patients nor investigators involved in rendering treatment or collecting data were aware of the treatment allocation. The moxifloxacin-containing gels and the placebo gel were indistinguishable in terms of consistency, color, smell, packaging, and labeling. There was only a difference in taste between the moxifloxacin-containing gels and the placebo gel. Because patients were unaware of the taste of the investigational substance and exposed to only one product, the masking of the treatment was not compromised. The results of the interim analysis were kept confidential and not conveyed to any of the investigators involved in rendering treatment or collecting data.

Statistical Analyses

The null hypothesis (i.e., that the reduction in PD between baseline and 12 weeks does not increase monotonically with increasing moxifloxacin concentrations) was tested using linear regression analysis. Pairwise comparisons of the three moxifloxacin gels with the placebo gel were performed using independent samples *t* tests. The global type I error rate was set to 0.025 (one-sided) and controlled for multiple testing by a priori ordering of hypotheses in the specified sequence, which accordingly, any lower-ranking null hypothesis could only be rejected after all higher-ranking null hypotheses were rejected.[28,29] The highest ranked null hypothesis was that the reduction in PD from baseline to 3 months does not increase monotonically with increasing moxifloxacin concentrations. Pairwise comparisons between moxifloxacin-containing gels and placebo were tested using lower-ranked hypotheses. For the interim analysis, a local boundary of 0.0102 (one-sided) had to be observed to reject the null hypothesis and an upper boundary of 0.5 was specified for stopping for futility. Statistical analyses were performed using a statistical program package[†††] on a personal computer system-[‡‡‡] running professional software.[§§§]

RESULTS

Participant Flow

A total of 65 patients were enrolled into the trial. Four patients were excluded before any treatment was rendered (three patients took systemic antibiotics and one patient moved away). Of the 61 patients who received the intended treatment, 60 completed the trial. One patient was withdrawn from the trial the day after the first treatment day because of a perforating duodenal ulcer. In addition, in three patients protocol violations were found to be critical with respect to the evaluation of treatment efficacy. These patients had received antiviral or antibiotic drugs or underwent dental treatments that may have influenced PD.

Baseline Data

At enrollment into the trial, patients were aged 31 to 68 years old, and 21 of the 57 patients were smokers (Table 1). Relevant medical conditions were reported in 36 patients. The most frequent medical conditions reported related to cardiovascular diseases, followed by allergic reaction and thyroid disorders. At baseline, mean (standard deviation [SD]) PD at the investigational sites were 6.8 (0.7) mm in the MOX 0.125, 6.5 (0.7) mm in MOX 0.4, 6.3 (0.5) mm in MOX 1.25, and 6.4 (0.7) mm in the control group ($P =$

††† SPSS for PC, v14.0.2, SPSS, Chicago, IL.
‡‡‡ AMD Athlon 64 X2, AMD, Sunnyvale, CA.
§§§ Microsoft Windows XP Professional, Microsoft, Redmond, WA.

Table 1.

Demographics of Patients Included in the Full Analysis Set

Variable	Control	MOX 0.125	MOX 0.4	MOX 1.25	*P* Value
n	15	16	15	11	
Male	4	7	7	6	0.55
Female	11	9	8	5	
Age in years (SD)	46 (9.4)	48.9 (7.2)	47.7 (10.1)	44 (9.3)	0.55
Active smoker	5	6	5	5	0.93

Statistical analysis for sex and smoking using χ^2 test *P* value (two-sided), and for age using one-way analysis of variance *P* value (two-sided).

Table 2.

Mean (SD) Baseline Values of Outcome Variables at Investigational Sites

Variable	Control	MOX 0.125	MOX 0.4	MOX 1.25
n	15	16	15	11
PD (mm)	6.4 (0.7)	6.8 (0.7)	6.5 (0.7)	6.3 (0.5)
GR (mm)	0.8 (0.9)	0.5 (0.4)	0.6 (0.5)	0.5 (0.4)
CAL (mm)	7.2 (1.1)	7.2 (0.9)	7.1 (0.8)	6.8 (0.9)
BOP (% of sites)	73.2 (24.2)	71.7 (25.3)	71.4 (26.7)	74.8 (18.1)
Suppuration (% of sites)	2.2 (5.9)	4.4 (13.1)	1.1 (4.3)	5.2 (12.3)
PI (% of sites)	64.8 (34.7)	70.4 (26)	61.1 (31.9)	63.5 (21.9)
FI (class)	0.8 (0.7)	1.1 (1)	0.7 (0.7)	0.8 (0.6)

0.3) (i.e., the baseline data for the primary outcome measure) (Table 2).

Numbers Analyzed

The analysis of treatment efficacy was by intention to treat and based on the full analysis data set. The analysis included 57 patients (16 in the MOX 0.125, 15 in the MOX 0.4, 11 in the MOX 1.25, and 15 in the control group) who were randomized and exposed to one of the investigational treatments and had any follow-up data after the administration of treatment available. In each patient, sites at the four investigational teeth with PD ≥5.4 at baseline were analyzed for efficacy testing. For safety assessment, all 61 patients were analyzed.

Random selection resulted in 35 (54.7%) of 64 in the MOX 0.125 group, 32 (53.3%) of 60 in the MOX 0.4 group, 24 (54.5%) of 44 in the MOX 1.25 group, and 20 (33.3%) of 60 of the investigational teeth in the control group being multirooted (molars and upper first premolars).

Outcomes and Estimations

Primary outcome variable. The null hypothesis, that PD reduction does not increase monotonically with higher moxifloxacin concentrations in the investigational gel, was not rejected and thus a linear trend for PD reduction with increasing moxifloxacin concentrations could not be confirmed ($P = 1.07$). The finding of no clear superiority of MOX 0.125 over placebo, and a descriptive superiority of the dosage of MOX 0.4 over MOX 1.25 were the primary reasons that the linear trend of PD reduction correlating with increasing moxifloxacin concentration could not be rejected. All ensuing pairwise comparisons of moxifloxacin concentrations versus placebo are to be interpreted as descriptive.

In all treatment groups, the PD decreased from baseline to 3 months, with the greatest reduction seen in patients treated with MOX 0.4 (1.5 mm; SD = 0.6 mm), followed by patients receiving MOX 1.25 (1.2 mm; SD = 0.4 mm), MOX 0.125 (1.1 mm; SD = 1.1 mm), and placebo (1 mm; SD = 0.6 mm). Pairwise comparisons of the three moxifloxacin concentrations to the control group showed significant superiority of MOX 0.4 ($P = 0.023$); the significance level MOX 1.25 was missed by a narrow margin ($P = 0.028$) (Fig. 1). There was no clear association between administered moxifloxacin doses and PD change. Patients treated with MOX 0.125 exhibited the greatest variance in PD reduction, whereas the outcome in patients who received MOX 1.25 was quite homogeneous, although it was found to rarely exceed an average reduction in PD of 1.5 mm. In the MOX 0.4 group, most patients showed a PD reduction by ≥1.5 mm; this explains why the largest average decrease was observed in this group (Fig. 2). The odds ratios for reducing mean initial PD of ≥5.4 mm at the investigational sites to mean PD <5 mm at 3 months compared to the control group was 0.92 (95% confidence interval [CI], 0.18 to 4.58) for MOX 0.125; 4.13 (95% CI, 0.88 to 19.27) for MOX 0.4; and 1.57 (95% CI, 0.29 to 8.42) for MOX 1.25.

Secondary outcome variables (Table 3). In all treatment groups, GR increased after therapy. The increase was greatest in the 0.4% moxifloxacin group

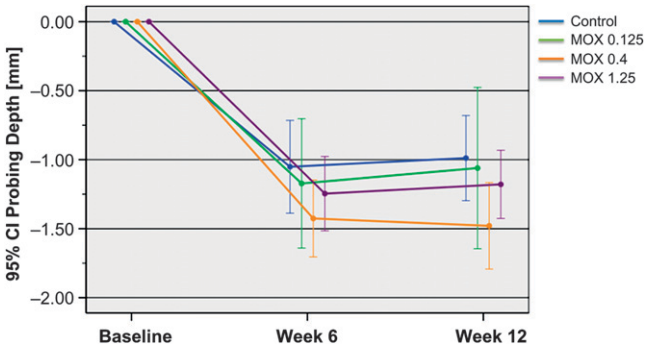

Figure 1.
Mean change in PD at investigational sites after treatment in each treatment group. Error bars indicate 95% confidence interval.

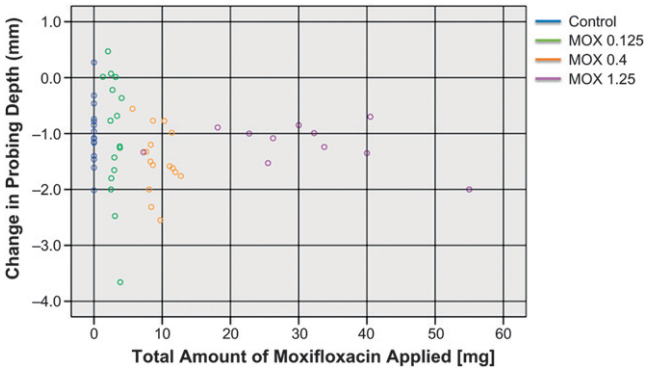

Figure 2.
Moxifloxacin concentration and total moxifloxacin dosages (milligrams) administered in relationship to PD reduction at investigational sites.

(0.7 mm; SD = 0.4 mm) and significantly different from the observed changes in the placebo group ($P<0.05$). There was a moderate correlation between the observed changes in GR and PD with a Pearson correlation value of $r = –0.58$. Mean gains in CAL ranged from 0.6 to 0.8 mm and mean BOP values decreased by 0.1% to 18.6%, with the greatest effect seen in the MOX 0.4 and MOX 1.25 groups. PI decreased slightly in all treatment groups, except the MOX 0.4 group, where it increased by 2%. No relevant changes were seen between baseline and 3 months in suppuration and furcation measurements. Differences among any of the test groups and the placebo group were not statistically different for CAL, BOP, suppuration, PI, and FI (Table 3).

Subgingival microbiota. *Porphyromonas gingivalis* showed the greatest trend toward reduced prevalence in all treatment groups both at 6 weeks and 12 weeks after therapy. *A. actinomycetemcomitans* decreased in the MOX 0.125 group and in the placebo group, but not in the other two groups. An increase in the prevalence of *S. intermedius* was observed in the placebo group. Changes in the prevalence of putative periodontal pathogens were not significantly different among treatment groups and placebo (Fig. 3).

Ancillary Analysis

Analysis of variance did not reveal any significant effects or interaction of smoking habit and extent of periodontitis with treatment outcome.

Adverse Effects

Before randomization and treatment, seven patients experienced adverse events. After receiving the first treatment, four patients in the placebo group had six adverse events, three patients in the MOX 0.125 group had seven events, two patients in the MOX 0.4 group had two events, and six patients in the MOX 1.25 group had seven events. The most frequent adverse events reported were gastrointestinal system disorders and resistance mechanism disorders. In 28 out of the 29 events reported, a causal relationship

Table 3.

Mean (SD) Change in Secondary Outcome Variables at Investigational Sites From Baseline to 3 Months

Variable	Control	MOX 0.125		MOX 0.4		MOX 1.25	
n	15	16	*P* Value	15	*P* Value	11	*P* Value
GR (mm)	0.4 (0.3)	0.4 (0.3)	0.49	0.7 (0.4)	0.01	0.4 (0.3)	0.47
CAL (mm)	–0.6 (0.5)	–0.7 (0.9)	0.40	–0.8 (0.4)	0.14	–0.8 (0.4)	0.15
BOP (% of sites)	–2.9 (22.8)	–0.1 (30.8)	0.61	–12.1 (25.3)	0.15	–18.6 (32.3)	0.09
Suppuration (% of sites)	–1.1 (4.3)	–2.8 (14.4)	0.33	–1.1 (4.3)	0.50	–5.2 (12.3)	0.12
PI (% of sites)	–13.2 (25.7)	–17 (47)	0.39	2.2 (27.7)	0.94	–2.2 (24.5)	0.86
FI (class)	0 (0)	–0.1 (0.4)	0.30	0 (0.4)	0.56	0 (0.4)	0.50

Positive numbers indicate an increase and negative numbers a decrease in value. Pairwise comparisons to control group performed using one-sided *t* test.

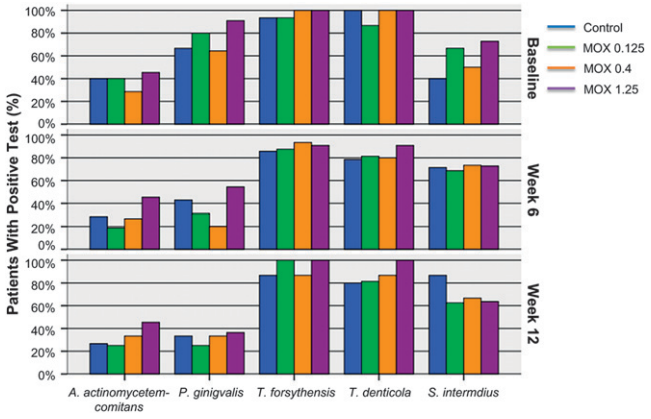

Figure 3.
Detection frequency of putative periodontal pathogens in patients at baseline, 6 weeks, and 12 weeks. Columns indicate fraction of patients harboring the tested bacterial species at least at one of the investigational sites from which subgingival plaque samples were taken.

with the study medication could be excluded. In one patient, who reported non-radiating left thoracic pain and nausea with concomitant hyperventilation 1 day after treatment, the causal relationship to the moxifloxacin treatment was considered to be unlikely. The event was non-serious and subsided on the same day without intervention.

In patients assessed for antibiotic susceptibility against moxifloxacin, two of the six patients in the MOX 0.125 group, all six patients in the MOX 0.4 group, and two of the three patients in the Control group harbored bacteria resistance against moxifloxacin at baseline. Resistance was most frequently found in *Prevotella loescheii* (six of 19 patients) and *C. rectus* (four of 19 patients). After treatment, new resistance to moxifloxacin was found in all but one patient (in the MOX 0.4 group) in all treatment groups. New resistance found in at least two patients treated with one of the moxifloxacin concentrations was demonstrated in *Actinomyces gerencseriae*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, *C. rectus*, and *Prevotella* spp. (Table 4).

Table 4.

Bacterial Species Displaying New Resistance Against Moxifloxacin After Treatment: Safety Analysis Set of 61 Patients

Organism	Control	MOX 0.125	MOX 0.4	MOX 1.25
Actinobacillus spp.	0	1	0	0
Actinomyces dentalis	1	1	0	0
Actinomyces gerencseriae	0	0	2	1
Actinomyces israelii	0	3	2	1
Actinomyces naeslundii	0	1	1	0
Actinomyces odontolyticus	0	2	0	1
Actinomyces spp.	1	0	1	0
Bifidobacterium spp.	0	0	0	1
Campylobacter concisus	1	0	0	0
Campylobacter gracilis	0	0	1	0
Campylobacter rectus	0	1	1	1
Eubacterium spp.	1	0	0	0
Gemella morbillorum	0	1	0	0
Leptotrichia buccalis	1	1	1	0
Leptotrichia spp.	0	0	0	1
Prevotella denticola	0	1	0	0
Prevotella loescheii	2	3	2	1
Prevotella marshii	1	0	0	0
Prevotella oralis	1	0	0	0
Prevotella spp.	0	1	1	0
Streptococcus mitis	0	1	0	0
Veillonella parvula	0	0	1	0

DISCUSSION

The relationship between moxifloxacin concentrations and in vitro bactericidal activities has been shown to follow a sigmoidal curve with the linear portion ranging from 0.01 of the minimal inhibitory concentrations (MIC) to 10 MIC.[30] The moxifloxacin concentrations used in this study (1,250, 4,000, and 12,500 mg/L) exceeded the MICs for putative periodontal pathogens by a factor of 10^2 to 10,[6,13-15,31] which may explain why no linear relationship was found between moxifloxacin concentration and PD reduction in this study. Nevertheless, because the a priori highest-ranked null hypothesis was rejected in that the reduction in PD from baseline to 12 weeks did not increase monotonically with increasing moxifloxacin concentrations, all pairwise comparisons between test and control treatments can only be accepted as confirmatory proof of efficacy and need to be interpreted descriptively.

The superiority of the 0.4% and 1.25% moxifloxacin gels over the placebo gel was a consistent finding in the statistical analysis, supporting the validity of moxifloxacin concentrations' efficacy in the adjunctive treatment of periodontitis. The mean effect size of the additional PD reduction of 0.5 mm between the

MOX 0.4 and placebo group is at the upper end of the meta-analytic averages ranging from 0.02 to 0.55 mm reported for local antimicrobial delivery systems for the treatment of periodontitis[32,33] and is more than twice the effect size reported for systemic administration of moxifloxacin.[17]

The robustness of the found efficacy of the 0.4% moxifloxacin gel is further supported by the fact that it could be demonstrated in a small sample size of 15 patients. In other locally delivered antimicrobial systems as an adjunct to scaling and root planing, effect sizes were only found to be significant at sample sizes as high as 42 to 232 subjects per group.[33]

Patients in the present study were stratified by smoking and extent of periodontal disease to mitigate the potential influence of these factors on the primary outcome variable, PD reduction. The finding that neither smoking nor initial disease extent had an interaction with PD reduction after therapy is in contrast with some previous reports, that showed less favorable outcomes in smokers compared to nonsmokers after scaling and root planing alone or in conjunction with locally delivered antibiotics.[34-39] However, an improved outcome after adjunctive locally delivered antibiotics compared to scaling and root planing alone has been a consistent finding among smokers.[39,40]

The investigational moxifloxacin gels seem to be safe as an adjunct to scaling and root planing in the treatment of periodontitis. No correlation or systematic relationship between adverse events and the various moxifloxacin gels was found in this study. However, it needs to be noted that rare possible adverse events may not be detected in this trial because the number of patients treated with a moxifloxacin gel was limited to 42.

There was no evidence demonstrating increased resistance to moxifloxacin caused by the investigational treatment. Antibiotic resistance of bacteria against moxifloxacin often occurs at concentrations within a mutant selection window, a bell-shaped function for AUC_{24}/MIC-dependent increase in MIC and resistance frequency.[41,42] Exposing *P. gingivalis* to subinhibitory fluoroquinolone concentrations has been shown to induce resistant mutants. *P. gingivalis* mutants exhibiting high resistance to ≥32 mg/L showed a serine-83 -> phenylalanine substitution in DNA gyrase, subunite A. Among the tested fluoroquinolones, moxifloxacin resulted in the lowest spontaneous mutation rate.[31] The mutant prevention concentrations of moxifloxacin are four times the MIC_{90} value for *Staphylococcus aureus*.[43] Although the mutant prevention concentrations of moxifloxacin for putative oral pathogens have not been determined, the moxifloxacin concentrations in the investigated gels at 10^2 to 10^6 MIC seem to be sufficiently high for mitigating the risk of resistance.

CONCLUSIONS

The results of this phase II trial indicate that in periodontal pockets of ≥5.4 mm in patients with moderate or severe chronic periodontitis, a single subgingival administration of a 0.4% or 1.25% moxifloxacin gel as an adjunct to scaling and root planing may result in the additional reduction of pocket probing compared to scaling and root planing alone. The investigated moxifloxacin gels seem to be safe. For a phase III trial, a 0.4% moxifloxacin gel concentration may be preferred because of its superior effect size without signs of an increased frequency or severity of adverse events.

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