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ORIGINAL ARTICLE

In vitro activity of taurolidine gel on bacteria associated with periodontitis

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Abstract
Objectives The purpose of this in vitro study was to determine the antimicrobial activity of two different taurolidine gel formulations in comparison with minocycline microspheres.
Methods Three percent taurolidine gel (TLG3) and 2 % taurolidine gel (TLG2) were compared to minocycline microspheres (MINO) against single bacterial species and a 12-species-mixture. The antimicrobial activity was proven by determination of minimal inhibitory concentrations (MICs), killing assays, after exposure of the antimicrobials as well as within a biofilm.
Results The MICs against the single species were between 0.5 and 2 mg/ml of taurolidine. MICs of the used mixed microbiota were 1.5 mg/ml (TLG3) and 4 mg/ml (TLG2). *Fusobacterium nucleatum* and *Porphyromonas gingivalis* were completely killed by 10 % TLG3 and TLG2 (equivalent to 3 and 2 mg/ml taurolidine) after 6 h. The mixture of 12 species was not completely killed by any of the test substances. Taurolidine gels showed a post-antimicrobial activity, however being less than that of MINO. On biofilms, taurolidine gels reduced concentration dependently the colony forming unit (CFU) counts (multi-species biofilms by 3.63 log10 after 100 % (30 mg/ml) of TLG3), reductions were 2.12 log10 after MINO (1000 μg/ml minocycline).
Conclusions Taurolidine gel formulations exert antimicrobial activity against bacteria associated with periodontal disease. Nevertheless, a complete elimination of biofilms seems to be impossible and underlines the importance of mechanical removal of biofilms prior to application of the antimicrobial.
Clinical relevance Taurolidine gels may represent a potential alternative for adjunctive topical antimicrobial treatment in periodontitis and infectious peri-implant diseases.

Keywords Taurolidine · Periodontal therapy · Antimicrobial activity · Biofilm

Introduction

The antimicrobial activity of taurolidine has been known for about 30 years. Taurolidine, a synthetic derivative of taurine, is described as an unstable molecule in aqueous solution; masked methanal is released which inactivates endotoxin [1]. The breakdown products methylol-taurultam and methylol-taurineamide are responsible for the antimicrobial action such as interaction with peptidoglycan [2]. Another breakdown product is taurine [2]. Taurine reacts with hypochlorous acid to taurine chloramine which can modify immune response [3].

The first indication for taurolidine was the prevention and treatment of peritonitis [4]. More recently, it has gained increased attention as a potential alternative in the treatment of dental infections. Results from ex vivo studies have provided evidence for the effectiveness of 2 % taurolidine in killing supragingival dental plaque [5] and, combined with mechanical treatment, in removal of biofilms from titanium specimens [6]. In comparison with chlorhexidine, taurolidine was less toxic to osteoblast-like cells and human gingival fibroblasts adhering to titanium surfaces [7].

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In a first in vitro study, we compared a 2 % taurolidine solution with a 0.1 % chlorhexidine digluconate solution in different dilutions [8]. The minimal inhibitory concentrations (MICs) of taurolidine were all below 5 % of the normally used concentration of that substance with the exception of *Candida albicans*. These findings confirm those from an earlier study which determined MIC values against seven oral plaque species, among them one *Fusobacterium nucleatum* and one *Prevotella intermedia* strain, and which has also revealed lower MIC values (dilution) for chlorhexidine compared to taurolidine [9].

Furthermore, our previous results have also indicated that taurolidine is active in a serum-rich environment in contrast to the activity of chlorhexidine that was dramatically decreased. Similar effects have been described before [10] and are of importance since gingival crevicular fluid contains up to 35 % of the albumin found in serum [11]. In a subsequent study, we compared taurolidine with minocycline against selected species associated with periodontitis and against a multi-species mixture consisting of 12 species [12]. The working concentration was chosen up to 50 % of the minimal bactericidal concentration (MBC) against the mixed population in planktonic stage. The results have also demonstrated a comparable or even better activity than those of minocycline.

When topical application of antibiotics is considered, the choice of controlled released devices is essential to overcome potential problems related to the high turnover of gingival crevicular fluid [13]. Usually, topical antibiotics are formulated as gels [14, 15] or microspheres [16] to be effective.

The purpose of the present study was to determine the antimicrobial effect of two taurolidine gel formulations in comparison with minocycline microsphere. The hypothesis was that taurolidine gel acts as antimicrobial like minocycline microspheres on microbial species associated with periodontitis and peri-implantitis. The antimicrobial activity was tested in killing assays, after exposure of the antimicrobials as well as within a biofilm.

Material and methods

Substances

As substances, a 3 % (*w*/*w*) taurolidine gel (TLG3 equivalent to 30 mg/g taurolidine) and a 2 % (*w*/*w*) taurolidine gel (TLG2 equivalent to 20 mg/g taurolidine; Geistlich Pharma AG, Wolhusen, Switzerland) were tested. As controls, minocycline microspheres (MINO, 1 mg; Arestin®, OraPharma Inc., North Bridgewater, NJ, USA), vehicles of TLG3 and TLG2 (Geistlich Pharma AG), and dH_2O were used. All substances including the controls were diluted in dH_2O containing 0.1 % *v*/*v* Tween 20. A twofold dilution series was always prepared from the 2 and 3 % taurolidine gels as well as from minocycline microspheres. Vehicles were only tested in an amount used equivalent to the highest tested concentration of taurolidine.

The used concentrations were based on the volume of a doxycycline gel to be placed into the pocket which was about 100 μl [17].

Microorganisms

The following bacterial strains were tested as single bacterial species: *Aggregatibacter actinomycetemcomitans* Y4, *Porphyromonas gingivalis* ATCC 33277, *F. nucleatum* ATCC 25586, and *Streptococcus gordonii* ATCC 10558. The mixed microbiota consisted of the following bacterial strains: *Streptococcus gordonii* ATCC 10558, *Actinomyces naeslundii* ATCC 12104, *F. nucleatum* ATCC 25586, *Campylobacter rectus* ATCC 33238, *Eubacterium nodatum* ATCC 33099, *Eikenella corrodens* ATCC 23834, *Prevotella intermedia* ATCC 25611, *Parvimonas micra* ATCC 33270, *Porphyromonas gingivalis* ATCC 33277, *Tannerella forsythia* ATCC 43037, *Treponema denticola* ATCC 35405, and *Aggregatibacter actinomycetemcomitans* Y4.

Before an experiment, all strains (except for *Treponema denticola* ATCC 35405) were precultivated on Schaedler agar plates (Oxoid, Basingstoke, UK) with 5 % sheep blood in an anaerobic atmosphere or with 5 % CO_2 (*Aggregatibacter actinomycetemcomitans* Y4 and *Streptococcus gordonii* ATCC 10558). *Treponema denticola* was maintained in modified mycoplasma broth (BD, Franklin Lake, NJ) added by 1 mg/ml glucose, 400 μg/ml niacinamide, 150 μg/ml spermine tetrahydrochloride, 20 μg/ml Na isobutyrate enriched with 1 g/ml cysteine, and 5 μg/ml cocarboxylase in anaerobic conditions.

Determination of the minimal inhibitory concentrations and minimal bactericidal concentrations

First, the MICs of taurolidine gels and MINO against the four single selected species as well as against the mixed population were determined.

Microbroth dilution technique was used for determination of MICs. After subcultivation of bacterial strains and checking of purity, a defined inoculum was added to Wilkins-Chalgren broth (Oxoid, Basingstoke, UK) containing defined concentrations of the antimicrobials (negative control, no agent as well as vehicles of the gels; positive control, MINO in twofold series dilution starting from 125 μg/ml minocycline). Taurolidine gels were tested in a twofold dilution series starting from 20 % of the gels (equivalent to 60 and 40 mg/ml of taurolidine, respectively). In part, 25 % inactivated serum (final concentration) was added. After an incubation time of 24 h, the growth of microbes was analyzed by visual checking of turbidity. MIC was determined as the lowest concentration

without visible turbidity of the broth and/or reduction of the initial inoculum of the colony forming units (CFU) on the agar plates. The MBC was the lowest concentration without any growth of the subcultivations on the agar plates (equivalent to a reduction by 99.9 % of the initial inoculum). Experiments were made at least in independent duplicates.

Killing

A defined inoculum of microorganisms (5×10^7) was prepared in nutrient media (Wilkins-Chalgren broth). The test substances were added. Taurolidine gels were tested in a twofold dilution series starting from final concentrations of 10 % of the gels (equivalent to 30 and 20 mg/ml of taurolidine, respectively) up to 1.25 % and of 1000 μg/ml up to 125 μg/ml minocycline (in MINO).

After 30 min and 1, 2, 4, 6, and 24 h of incubation, the numbers of viable bacteria were determined by enumeration of colony forming units (CFU). (In case of the mixed microbiota, only the total numbers of CFU were counted.)

Substantivity (growth inhibition after exposure)

Microorganisms (about 10^8/ml) were exposed to antimicrobials in 500 μl of Wilkins-Chalgren broth for 2 h. Taurolidine gels and MINO were tested in the same concentrations as described for killing assays. After that, the suspensions were centrifuged for 10 min at 5000*g*. The supernatant was removed and 5 ml of nutrient broth (Wilkins-Chalgren broth) was added. The CFU counts were determined after 1, 2, 6, and 24 h.

Activity of taurolidine gel against bacteria within biofilms

In these experiments, *Aggregatibacter actinomycetemcomitans* Y4 and *Porphyromonas gingivalis* ATCC 33277 were used to form a single-species biofilm. In addition, a multi-species biofilm consisting of the 12 species was established. First, the wells of 24-well plates were covered with 100 μl of 25 %*v*/*v* inactivated human serum/well for 1 h. Then, 1 ml of bacterial suspension was added. The medium was Brain-Heart Infusion broth (Oxoid Ltd.) with 5 % blood (and 5 μg/ml cocarboxylase for the mixed population). The 24-well plates were incubated in the appropriate atmosphere. After 60 h, the medium was carefully exchanged. In case of the mixed biofilm, *Porphyromonas gingivalis* ATCC 33277, *Tannerella forsythia* ATCC 43037, and *Treponema denticola* ATCC 35405 were again added to the nutrient medium before application to the wells. The renewed addition of selected bacterial strains guaranteed a sufficient number of these species within the biofilms.

After an additional incubation for 48 h, the medium was removed carefully, and 100 μl of the gels in different concentrations and MINO in suspension were added to the biofilm for 1 h in the appropriate incubation. The gels were prepared on sterile paper discs, thus allowing testing up to 100 % of the gel formulations. However, minocycline microspheres could not be adequately dissolved; here the highest tested concentration was only 1000 μg/ml. After 1 h, 900 μl of Wilkins-Chalgren broth supplemented with 5 % sheep blood (and 5 μg/ml cocarboxylase for the mixed population) was added. This simulates in part in vivo conditions, where a diluting effect of subgingivally applied antimicrobials can be assumed. The plates were incubated in the appropriate atmosphere overnight (18 h). Then, the medium was removed. Finally, the biofilm was carefully scraped and mixed by pipetting, and CFU were enumerated after serial dilutions, spreading of each 25 μl on agar plates, and incubation for 48 h (*Aggregatibacter actinomycetemcomitans* Y4) and 7 days (*Porphyromonas gingivalis* ATCC 33277 and mixed microbiota).

Moreover, the potential inhibition of biofilm formation by the antimicrobials was tested. For this purpose, wells were covered with 100 μl of the antimicrobials (12.5 % of the gels as well as 125 μg/ml minocycline) first. Thereafter, the biofilm was formed as described above for 24 and 48 h. At these time points, the CFU counts were determined.

The experiments were made in independent triplicates.

Results

Minimal inhibitory and minimal bactericidal concentrations

The MICs and MBCs of taurolidine against the four single species were between 0.5 and 2 mg/ml and between 1 and 3 mg/ml of taurolidine when testing both taurolidine gel formulations. When adding 25 % serum, the MICs were equal or one step higher. The used mixed microbiota was less sensitive to taurolidine; MIC was 1.5 mg/ml (TLG3) and 4 mg/ml (TLG2); the MBCs were 4–6 mg/ml. When adding serum, MICs were similar, whereas taurolidine was not bactericidal within the tested concentrations of 4 and 6 mg/ml. The vehicles did not show any growth inhibitory effect. Sensitivity of the included stains varied against minocycline; the highest MICs and MBCs were 63 μg/ml. The results are presented in Tables 1 and 2.

Considering the concentration of taurolidine in the formulations, the results mean that the MICs of the TLG3 formulation were up to 5 % of the formulation except for the mixed microbiota with serum (10 %). MBCs of TLG3 did not exceed 10 % of the formulation for the single species whereas it was higher than 20 % of the formulation against the mixed microbiota with serum. The MICs of the TLG2 formulation were up to 10 % of the formulation except for the mixed microbiota

Table 1 Minimal inhibitory concentration (MIC) values of taurolidine within the 3 % (TLG3) and the 2 % (TLG2) formulations as well as of minocycline within the minocycline microspheres (MINO) against selected oral species and a bacterial mixture consisting of 12 different species associated with periodontitis

	mg/ml of taurolidine (TLG3)		mg/ml of taurolidine (TLG2)		μg/ml minocycline (MINO)	
	w/o serum	with 25 % serum	w/o serum	with 25 % serum	w/o serum	with 25 % serum
S. gordonii ATCC 10558	1.5	1.5	2	2	8	4
A. actinomycetemcom. Y4	0.75	1.5	0.5	1	31	31
P. gingivalis ATCC 33277	0.75	1.5	0.5	2	31	31
F. nucleatum ATCC 25586	1.5	1.5	1	1	16	31
12 species (mixed)	1.5	3	4	4	63	31

with serum (20 %). MBCs of TLG2 were up to 20 % of the formulation for the single species; it was higher than 20 % of the formulation against the mixed microbiota with serum.

Killing of planktonic bacteria

F. nucleatum ATCC 25586 and *Porphyromonas gingivalis* ATCC 33277 were completely killed by the highest used concentrations of 10 % TLG3 and TLG2 (equivalent to 3 and 2 mg/ml taurolidine) after 6 h. In case of *Streptococcus gordonii* ATCC 10558 and *Aggregatibacter actinomycetemcomitans* Y4, the respective times were 2 and 4 h (TLG3) and 4 and 6 h (TLG2), suggesting a higher sensitivity of these strains to the antimicrobials' killing. After 2 h, minocycline microspheres in the concentration of 1000 μg/ml killed all tested single strains except for *Aggregatibacter actinomycetemcomitans* Y4 which was eradicated after 6 h by all tested concentrations of minocycline microspheres.

Focusing on the mixture of 12 selected bacterial species associated with periodontitis, neither taurolidine gels nor MINO was able to kill completely all bacteria; in comparison with the control, the reductions were 3.61 log10 CFU for 10 % TLG3 (3 mg/ml taurolidine), 2.89 log10 CFU for 10 % TLG2 (2 mg/ml taurolidine), and 4.77 log10 CFU for MINO (100 μg/ml minocycline). The vehicles of both gel formulations did not influence the growth of the single strains and the bacterial mixture (Fig. 1).

Post-antibiotic (depot) effect

Bacteria were exposed to the antimicrobial formulations for 2 h; thereafter, the media were replaced by a medium free of antimicrobial agents. This procedure should show a possible depot effect of the antimicrobials. Immediately after exposure to 10 % (3 mg/ml taurolidine) of the TLG3 formulations, no viable *Porphyromonas gingivalis* ATCC 33277 and *F. nucleatum* ATCC 25586 were found, 10 % (2 mg/ml taurolidine) of the TLG2 eradicated *F. nucleatum* ATCC 25586 immediately, whereas *Porphyromonas gingivalis* ATCC 33277 was killed only after 24 h. No complete killing was found in case of S. *gordonii* ATCC 10558 and *Aggregatibacter actinomycetemcomitans* Y4. MINO in its tested highest concentrations eradicated all viable bacteria at the latest after 6 h. Vehicles in part slightly decreased transitory the growth of bacteria, e.g., the TLG2 vehicle reduced the CFU counts of *Aggregatibacter actinomycetemcomitans* Y4 by about 2 log10 CFU immediately after exposure and 1 h of growth (Fig. 2).

The antimicrobials reduced CFU counts of the mixture in a concentration-dependent manner but were unable to kill

Table 2 Minimal bactericidal concentration (MBC) values of taurolidine within the 3 % (TLG3) and the 2 % (TLG2) formulations as well as of minocycline within the minocycline microspheres against selected oral species and a bacterial mixture consisting of 12 different species associated with periodontitis

	mg/ml of taurolidine (TLG3)		mg/ml of taurolidine (TLG2)		μg/ml minocycline (MINO)	
	w/o serum	with 25 % serum	w/o serum	with 25 % serum	w/o serum	with 25 % serum
S. gordonii ATCC 10558	3	3	4	2	63	31
A. actinomycetemcom. Y4	1.5	1.5	0.5	1	63	63
P. gingivalis ATCC 33277	1.5	3	1	2	63	31
F. nucleatum ATCC 25586	1.5	1.5	2	2	16	31
12 species (mixed)	6	>6	4	>4	63	63

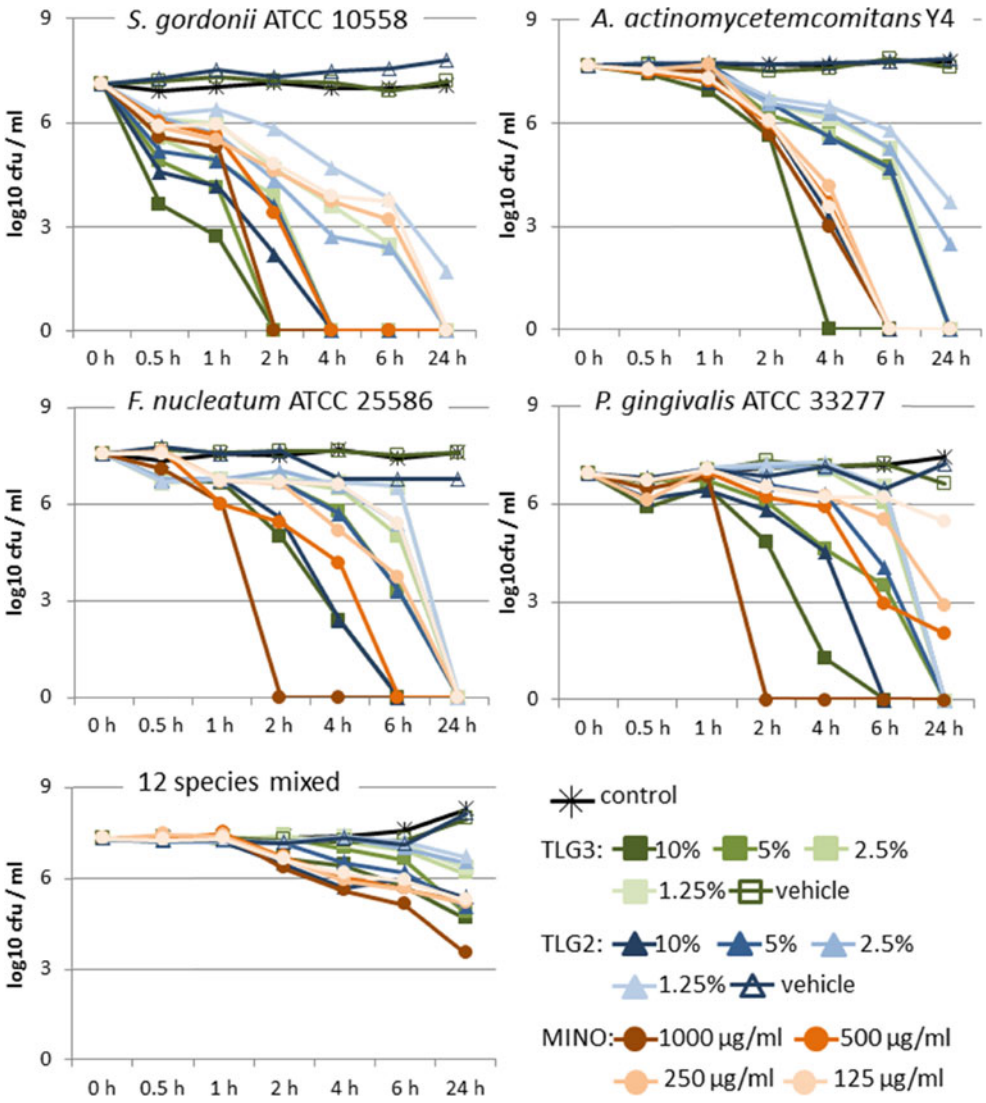

Fig. 1 Killing of selected oral species and a bacterial mixture consisting of 12 species associated with periodontitis by taurolidine gel formulations and minocycline microspheres

completely bacteria after an exposure of 2 h. The CFU count reductions were 2.34 log10 CFU immediately after exposure to 10 % of TLG3 (3 mg/ml taurolidine), 1.79 log10 CFU after 10 % of TLG2 (2 mg/ml taurolidine), and 2.63 log 10 CFU after MINO (1000 µg/ml minocycline). After 24 h, the respective values were 1.93 log10 CFU for 10 % of TLG3 (3 mg/ml taurolidine), 1.94 log10 CFU for 10 % of TLG2 (2 mg/ml taurolidine), and 4.89 log 10 CFU after MINO (1000 µg/ml). The vehicles of the taurolidine gels did not influence the bacterial growth (difference always ≤0.5 log10 CFU) (Fig. 2).

Activity on biofilms

On 4.5-day-old biofilms, taurolidine acted clearly concentration dependently; the TLG3 formulations were more active than TLG2. In single-species biofilms, *Aggregatibacter actinomycetemcomitans* Y4 was not detectable after exposing to 25–100 % (7.5–30 mg/ml taurolidine) of the TLG3 and 50–100 % (10–20 mg/ml taurolidine) of the TLG2; the CFU counts of the *Porphyromonas gingivalis* ATCC 33277 biofilms were reduced after application of 50 and 100 % of the TLG3 and 100 % of the TLG2 by about 6.5 log10 CFU. Minocycline in a concentration of 1000 µg/ml killed *Aggregatibacter actinomycetemcomitans* Y4 within biofilm, but no activity was found on *Porphyromonas gingivalis* ATCC 33277 biofilms. The reduction of the CFU counts in the multi-species biofilms was 3.63 log10 CFU after application of 100 % of TLG3 taurolidine gel and 2.62 log10 CFU after 100 % of TLG2 taurolidine gel. MINO (1000 µg/ml minocycline) reduced the CFU by 2.12 log10 (Fig. 3).

The biofilm formation of *Aggregatibacter actinomycetemcomitans* Y4 and of *Porphyromonas gingivalis* ATCC 33277 was clearly disrupted after coating the plates with 12.5 % of taurolidine gels (3.75 and 2.5 mg/ml taurolidine) and MINO (125 µg/ml minocycline). Interestingly, the vehicle of the TLG2 gel decreased the adhesion of bacteria (differences after 48 h are 0.99 log10 CFU for

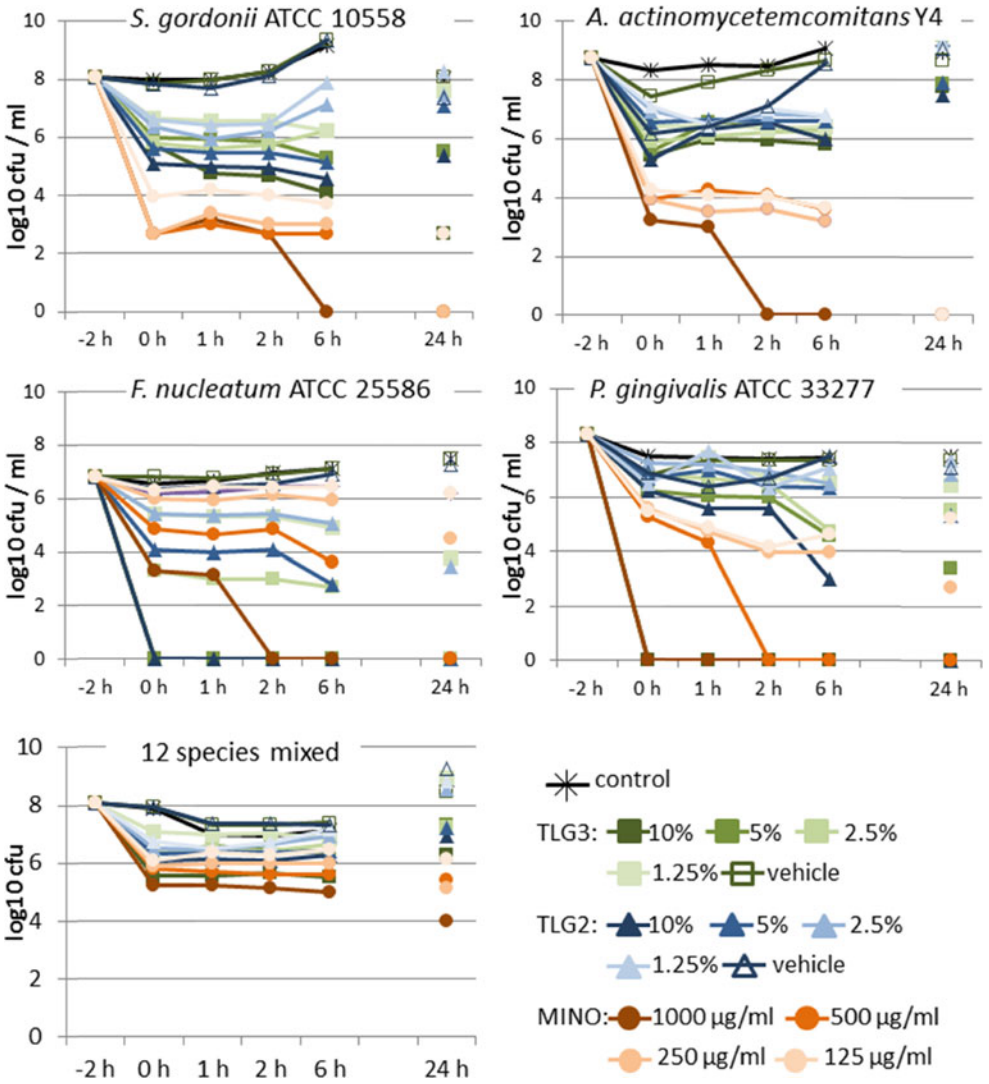

Fig. 2 Numbers of viable bacteria of selected oral species (determined by colony forming unit counts) after 2 h of exposure to taurolidine gel formulations and minocycline microspheres and subsequent cultivation in antimicrobial free media

Aggregatibacter actinomycetemcomitans Y4 biofilms, 4.50 log10 CFU for *Porphyromonas gingivalis* ATCC 33277 biofilms, and 1.17 log10 CFU for multi-species biofilm). TLG2 was more preventive than TLG3 on *Porphyromonas gingivalis* ATCC 33277 biofilm formation (reduction 8.05 vs. 5.91 log10 CFU). Most active was MINO (reduction 9.16 log10 CFU). TLG2 and TLG3 did not influence the formation of the multi-species biofilm; in contrast, MINO decreased the adhesion by 4.44 log10 CFU after 24 h and by 3.98 log10 CFU after 48 h (Fig. 4).

Discussion

In the present study, the in vitro activity of two taurolidine gel formulations was compared with those of minocycline microspheres. Taurolidine solutions have been successfully proven as a catheter-lock agent to prevent catheter-related bloodstream infections in patients on home parenteral nutrition [18, 19], in central venous catheters in children with cancer [20], and in hemodialysis patients [21]. By designing our study, we aimed to gain more insight on the potential use of taurolidine as alternative to commonly used topical antimicrobials, e.g., minocycline. Minocycline microspheres have been introduced in periodontal therapy for more than 10 years. Its efficacy was demonstrated in a large post-marketing study including 2805 patients in dental practices [22], and its adjunctive use to nonsurgical periodontal therapy has been shown to yield additional clinical benefits (i.e., probing depth reduction) compared with nonsurgical periodontal therapy alone [23]. The potential clinical benefit of minocycline microspheres as an adjunctive to nonsurgical periodontal therapy was also confirmed in a recent systematic review [24].

Taurolidine in gel formulations showed favorable MIC values against the included single species with and without serum and were in the range or slightly higher tested before for the 2 % solution [8, 9] meaning that the additives of the gel do not

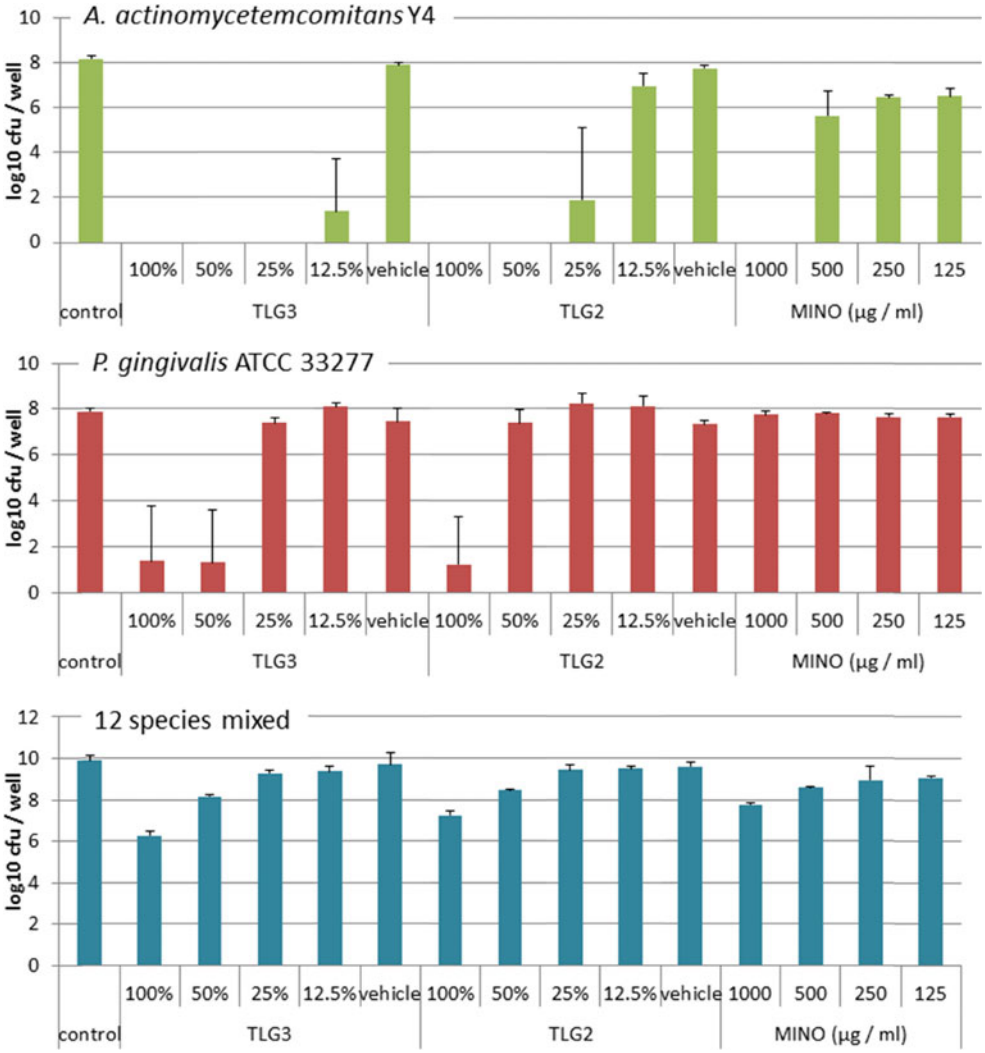

Fig. 3 Effect of taurolidine gels and minocycline microspheres on 4.5-day-old single-species biofilms of *Aggregatibacter actinomycetemcomitans* Y4, *Porphyromonas gingivalis* ATCC 33277, and on 4.5-day-old multi-species biofilms of 12 different species associated with periodontitis. Antimicrobials were added for 1 h and thereafter diluted 1:10, and well plates were incubated for 18 h, before CFU counts were determined

interfere with the antimicrobial activity of taurolidine. Although gel formulations may inhibit washout of the drug by the high turnover of gingival crevicular fluid [13], a flow-out and a decreasing concentration of the antimicrobial can be assumed. Following antimicrobial activity below the MIC in the formulations is a sine qua non condition. The MICs were less or equal than 10 % of the available concentration within gels except for the mixed population in 25 % serum where the MICs were 20 % of the 2 % taurolidine gel (4 mg/ml taurolidine).

As shown recently a catheter-lock solution a 2 % taurolidine solution was more bactericidal than the commonly used antibiotic vancomycin against *Staphylococcus aureus* [25]. In our present study, concentrations up to 10 % of the formulations were tested in the killing assays and in determinations of a post-antimicrobial activity. This was due in part to the insolubility of the compounds. On the other hand, this is more adapted to the in vivo situation in oral cavity where a diluting effect can be expected very fast. A clear concentration-dependent activity for taurolidine was confirmed. But, in contrast to our previous results on a 2 % solution [12], the mixed population was not eliminated by the gel formulations. Elimination of multi-species mixtures per se might be critical. Although tested in high concentrations, minocycline microspheres did not result in a favorable outcome in comparison with taurolidine gels.

Post-antimicrobial activity is needed for topical applications of antimicrobials because a fast diluting effect can be expected. For example, gingival crevicular fluid in periodontitis patients has a volume of up to 1.5 µl and a turnover of up to 44 µl/h [13]. Although taurolidine gels were able to retard growth of selected single-species and the multi-species mixture, they were less active than minocycline microspheres on bacteria when the exposure to the antimicrobial was stopped.

In dentistry and in particular in periodontal therapy, any antimicrobial should exert activity on biofilms. Models of single-species and of a 12-species biofilm were tested. In vivo subgingival biofilm consists of hundreds of different taxa [26]; bacteria co-aggregate, communicate, and transfer DNA [27].

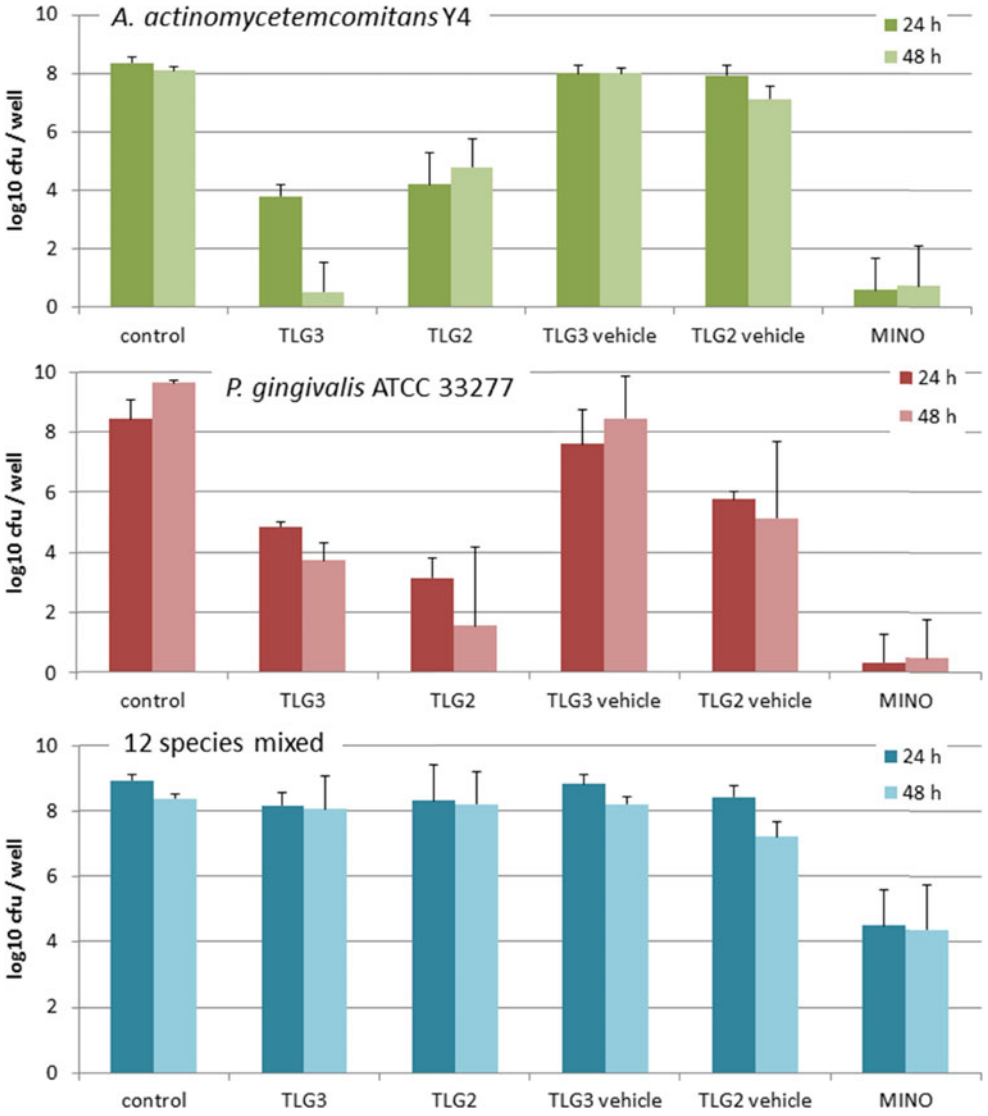

Fig. 4 Effect of taurolidine gels and minocycline microspheres on formation of single-species biofilms of *Aggregatibacter actinomycetemcomitans* Y4, *Porphyromonas gingivalis* ATCC 33277, and of multi-species biofilms of 12 different species associated with periodontitis. After coating surface with 12.5 % of taurolidine gels (3.75 and 2.5 mg/ml of taurolidine) and 125 μg/ml of minocycline, biofilms were formed for 24 and 48 h

Antimicrobials are not as active as against planktonic bacteria which are associated with limited growth, metabolic activity, and a polymer matrix around microcolonies [28].

Taurolidine in gel formulations reduced the CFU counts of an existing single-species biofilm clearly. *Aggregatibacter actinomycetemcomitans* Y4 biofilms were eradicated; reductions were up to 7 log10 CFU when acting on *Porphyromonas gingivalis* biofilms. MINO was not as active on *Porphyromonas gingivalis* biofilms. Minocycline in comparison with chlorhexidine decreased activity of *Porphyromonas gingivalis* biofilm less, but it was more reducing than metronidazole [29]. In other in vitro experiments, 2 % taurolidine solution reduced viability by 5–6 log10 CFU in *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* biofilms [30]. However, confirming a recent report [31], activity of antimicrobials is limited on a multi-species biofilm. Here, only the 100 % concentration of a 3 % taurolidine gel was able to reduce the CFU counts by more than 3 log10. In dental ex vivo biofilm models, the activity of taurolidine was less than that of chlorhexidine, but change of vitality was tested already after 2 min of exposure [5]; however, in a study with 15 volunteers, the findings were similar [32].

Very recent in vitro tests of taurolidine containing catheter-lock solutions have shown a biofilm preventing activity up 0.25 % [33]. In our study, a biofilm formation was inhibited but not completely prevented by taurolidine gels and by MINO. This may be related to the experimental design. Surfaces were coated; thereafter, bacterial suspension was added. First, the surface might be the reservoir of the antimicrobial; later, an equal distribution in the medium (concentration about 0.04 %) can be assumed. In comparison, taurolidine in solution with a final concentration of 0.1 % was more active in inhibiting biofilm formation [12]. This underlines again the necessity of a high concentration of taurolidine over a longer time period. Although not acting bactericidal, the vehicle of the TLG2

formulation seemed to have a slightly inhibiting effect. This may be associated to menthol as one excipient of the vehicle.

In this study, antimicrobial activities were investigated. A general problem in using antimicrobials is the development of resistance. Increase of MIC values has been demonstrated in vitro for minocycline [34]. In vivo, an increase of percentage of tetracycline-resistant streptococci was found after topical use of minocycline [35]. The mode of action of taurolidine makes resistance unlikely [25]. After a long-term use of taurolidine as a catheter-lock solution, no microbial adaption was found [18].

Other, not yet studied, properties of taurolidine might be also advantageous in periodontal therapy. In an animal model, wound healing was not impaired after intravenous and intraperitoneal application of a 3 % taurolidine solution [36]. Moreover, it appears that taurolidine has certain immunomodulatory effects evidenced by inhibiting ex vivo expression of inflammatory cytokines (e.g., interleukin(IL)-6 and tumor necrosis factor α) and increasing the release of anti-inflammatory cytokine IL-10 [37].

Conclusion

Both taurolidine-containing gels are active against bacterial species associated with periodontitis, even within biofilms. The clear concentration-dependent activity underlines the necessity of a high concentration being active for a defined time at the site of the infection. Additives to the gels (vehicles) may support a depot or anti-adhesive effect of the antimicrobial. Our results indicate to continue with the higher concentrated taurolidine gel (3 %*w*/*v*) in follow-up in vitro studies.

The tested taurolidine gels have potential as an adjunctive antimicrobial treatment in periodontitis and warrant further evaluation in in vitro and in clinical trials. Nevertheless, complete elimination of multi-species biofilms by taurolidine gels seems to be impossible and underlines the importance of a mechanical removal of biofilms prior to application of the antimicrobial.

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Conflict of interest This study was funded by Geistlich Pharma AG, Wolhusen, Switzerland. Jürg Zumbrunn is an employee of Geistlich Pharma AG. The other authors declare that they have no conflict of interest.

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