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In-vitro activity of taurolidine on single species and a multispecies population associated with periodontitis

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ABSTRACT

The antimicrobial activity of taurolidine was compared with minocycline against microbial species associated with periodontitis (four single strains and a 12-species mixture). Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs), killing as well as activities on established and forming single-species biofilms and a 12-species biofilm were determined. The MICs of taurolidine against single species were always 0.31 mg/ml, the MBCs were 0.64 mg/ml. The used mixed microbiota was less sensitive to taurolidine, MIC and the MBC was 2.5 mg/ml. The strains and the mixture were completely killed by 2.5 mg/ml taurolidine, whereas 256 μg/ml minocycline reduced the bacterial counts of the mixture by 5 log10 colony forming units (cfu). Coating the surface with 10 mg/ml taurolidine or 256 μg/ml minocycline prevented completely biofilm formation of *Porphyromonas gingivalis* ATCC 33277 but not of *Aggregatibacter actinomycetemcomitans* Y4 and the mixture. On 4.5 d old biofilms, taurolidine acted concentration dependent with a reduction by 5 log10 cfu (*P. gingivalis* ATCC 33277) and 7 log10 cfu (*A. actinomycetemcomitans* Y4) when applying 10 mg/ml. Minocycline decreased the cfu counts by 1–2 log10 cfu independent of the used concentration. The reduction of the cfu counts in the 4.5 d old multi-species biofilms was about 3 log10 cfu after application of any minocycline concentration and after using 10 mg/ml taurolidine. Taurolidine is active against species associated with periodontitis, even within biofilms. Nevertheless a complete elimination of complex biofilms by taurolidine seems to be impossible and underlines the importance of a mechanical removal of biofilms prior to application of taurolidine.

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1. Introduction

Periodontitis is a bacterially induced chronic inflammatory disease, and an imbalance of innate immune-defence system markedly contributes to the destruction of the periodontium [1]. Microorganisms are organized in biofilms, the subgingival biofilms consist of hundreds of species. Bacteria more present in periodontitis than in periodontal health are *Treponema denticola*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans* and several others [2]. *P. gingivalis* a gram-negative anaerobe bacterium is postulated to be a keystone pathogen in developing periodontal disease [3]. The most important virulence factor of *A. actinomycetemcomitans* is being a leukotoxin able to cause imbalance in the host inflammatory response [4].

Non-surgical removal of the microbial deposits, the mechanical root debridement by scaling and root planing (SRP) is considered as a standard of cause-related periodontal therapy. There is a substantial evidence that during supportive periodontal therapy, the progression of periodontitis can be controlled thorough mechanical plaque removal performed by the patient and the therapist eventually in conjunction with the use of antimicrobials [5]. The application of different antimicrobials adjunctive the mechanical removal of deposits by SRP has been tested. As antimicrobials, chlorhexidine, azithromycin, metronidazole, doxycycline, minocycline and tetracycline were used [6]. Results from a systematic review have indicated that the subgingival application of tetracycline fibres and of sustained released doxycycline and minocycline demonstrated significant benefit in clinical outcome (probing depth reduction) [6].

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An alternative not tested yet in periodontal therapy might be taurolidine. The antimicrobial activity of taurolidine has been known for about 20 years. Taurolidine is described as an unstable molecule in aqueous solution, masked methanol molecules are released which inactivate endotoxin [7]. Further derivatives are responsible for the antimicrobial action such as interaction with peptidoglycan [8]. First it was used in prevention and treatment of peritonitis [9]. The substance may be also for interest in dentistry. Recently it has been shown in an ex vivo-model that 2% taurolidine is effective in killing supragingival plaque [10]. In an in vitro-study, we compared a commercially available 2% taurolidine solution with a 0.1% chlorhexidine digluconate solution [11]. The minimal inhibitory concentrations (MICs) of taurolidine were all below 1 mg/ml of taurolidine (equivalent to 5% of the normally used concentration of that substance) with the exception of *Candida albicans*. This confirms an earlier study which determined MIC values against seven oral plaque species, among them one *Fusobacterium nucleatum* and one *Prevotella intermedia* strain [12]. Further our results clearly indicated, that taurolidine was active in a serum rich environment. Contrary the activity of chlorhexidine was dramatically decreased [11]. These findings are important, because gingival crevicular fluid contains up to 35% of the albumin found in serum [13].

The purpose of this in-vitro-study was to determine the antimicrobial activity of taurolidine in comparison with minocycline on microbial species associated with periodontitis within a mixed planktonic population and within a biofilm.

The hypothesis was that taurolidine acts as antimicrobial as minocycline on a mixed microbiota associated with periodontitis. The antimicrobial activity was to be proven in killing assays, after exposure of the antimicrobials as well as within a biofilm.

2. Material and methods

2.1. Substances

Test substances were taurolidine in a 2% w/v solution (Geistlich TauroSept®, Geistlich Pharma AG, Wolhusen, Switzerland) and minocycline (Sigma–Aldrich, St. Louis, MO, USA). After determination of MICs and minimal bactericidal concentrations (MBCs) the final concentrations in the assays were 2.5, 5 and 10 mg/ml for taurolidine and 64, 128 and 256 μg/ml minocycline. Distilled water was used as a negative control. The tested concentrations were chosen in accordance with determined MBC values.

2.2. Microorganisms

The following bacterial strains were tested as single bacterial species: *A. actinomycetemcomitans* Y4, *P. gingivalis* ATCC 33277, *F. nucleatum* ATCC 25586 and *Streptococcus gordonii* ATCC 10558. The mixed microbiota consisted of the following bacterial strains: *S. gordonii* ATCC 10558, *Actinomyces naeslundii* ATCC 12104, *F. nucleatum* ATCC 25586, *Campylobacter rectus* ATCC 33238, *Eubacterium nodatum* ATCC 33099, *Eikenella corrodens* ATCC 23834, *P. intermedia* ATCC 25611, *Parvimonas micra* ATCC 33270, *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037, *T. denticola* ATCC 35405 and *A. actinomycetemcomitans* Y4. Before an experiment, all strains (except for *T. 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 (*A. actinomycetemcomitans* Y4 and *S. gordonii* ATCC 10558). *T. 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.

2.3. Determination of the minimal inhibitory concentrations and minimal bactericidal concentrations

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

Taurolidine was tested in a two-fold dilution series starting from 10 mg/ml and minocycline from 256 μg/ml (final concentrations) with microbroth-dilution technique by using 96-well-microtiter plates. The test medium was double-concentrated Wilkins Chalgren broth (Oxoid) by adding 1:1 of the antimicrobial in an aqueous solution. MIC was determined as the lowest concentration without visible turbidity of the broth. 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).

2.4. Killing

A defined inoculum of microorganisms (5×10^6) was prepared in doubled concentrated nutrient media (Wilkins Chalgren broth). The test substances were added in a ratio 1:1. After 15 min, 30 min, 1 h, 2 h, 6 h as well as 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.)

2.5. Substantivity (growth inhibition after exposure)

Microorganisms (10^8/ml) were exposed to antimicrobials in 3 ml of Wilkins-Chalgren broth for 2 h. After that, the suspensions were centrifuged 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 h, 2 h, 4 h, 6 h and 24 h.

All experiments were be made in independent replicates.

2.6. Activity against bacteria within biofilms

In these experiments *A. actinomycetemcomitans* Y4, *P. gingivalis* ATCC 33277 were used to form a single species biofilm. In addition, a multispecies 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 *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037 and *T. 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 [14].

After an additional incubation for 48 h, the medium was removed carefully, 100 μl of the antibiotic dilutions mixed with 100 μl of doubled concentrated Wilkins Chalgren broth were added. After 1 h, 800 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, mixed by pipetting and cfu were enumerated after serial dilutions, spreading of each 25 μl on agar

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