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ORIGINAL ARTICLE

Antibacterial effect of taurolidine (2%) on established dental plaque biofilm

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Abstract Preliminary data have suggested that taurolidine may bear promising disinfectant properties for the therapy of bacterial infections. However, at present, the potential antibacterial effect of taurolidine on the supragingival plaque biofilm is unknown. To evaluate the antibacterial effect of taurolidine on the supragingival plaque biofilm using the vital fluorescence technique and to compare it with the effect of NaCl and chlorhexidine (CHX), 18 subjects had to refrain from all mechanical and chemical hygiene measures for 24 h. A voluminous supragingival plaque sample was taken from the buccal surfaces of the lower molars and wiped on an objective slide. The sample was then divided into three equal parts and mounted with one of the three test or control preparations (a) NaCl, (b) taurolidine 2% and (c) CHX 0.2%. After a reaction time of 2 min, the test solutions were sucked of. Subsequently, the plaque biofilm was stained with fluorescence dye and vitality of the plaque flora was evaluated under the fluorescence microscope (VF%). Plaque samples treated with NaCl showed a mean VF of 82.42±6.04%. Taurolidine affected mean VF with 47.57±16.60% significantly ($p<0.001$, paired t test). The positive control CHX showed the lowest mean VF values (34.41±14.79%; $p<0.001$ compared to NaCl, $p=0.017$ compared to taurolidine). Taurolidine possesses a significant antibacterial effect on the supragingival plaque biofilm which was, however, not as pronounced as that of CHX.

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Introduction

The bacterial biofilm is considered as the main etiological factor for gingivitis and periodontitis [1, 2]. However, a large body of evidence suggests that none of the currently available instrumentation techniques are effective in completely removing the supra- and subgingival calculus and the bacterial biofilm. These limitations are mostly attributed to the complex anatomy of the teeth and limitations due to the size of instruments or the invasion of periodontal pathogens into the surrounding soft tissues or a recolonisation of pocket groom other sites or intraoral niches [3].

Modern concepts for the prevention and therapy of biofilm associated infections, e.g. caries, endodontal infections, gingivitis and periodontitis are not solely based on mechanical removal but also on the use of adjuvant substances with antibacterial properties (for review, see [4]). Efficient oral antimicrobial substances should prevent or at least reduce plaque growth. Numerous reviews describe both clinical and antibacterial effects of substances (e.g. [4]). In dentistry, chlorhexidine (CHX, in concentrations of 0.1–0.2%) can still be considered as the golden standard [4–7]. Numerous studies support its efficiency against plaque and gingivitis both for prevention and therapy. It can be used as an adjuvant to mechanical measurements but also as a chemical toothbrush, when mechanical hygiene can or should not be performed.

However, CHX has some well-known side effects; the most common is yellow brown or black staining of teeth, tongue and restorations [8]. Although these are mostly removable by professional tooth cleaning and should not lead to decline when indicated, more and more patients are

concerned by this impairment. CHX with "anti-staining" agents could not yet prove their efficacy [7, 9].

A second disadvantage (and a general disadvantage of antiseptics) is a possible toxicity to host tissues due to its unspecific mechanism of action (pantoxicity), which is controversially discussed: A cytotoxic effect of CHX has been shown in vitro for blood cells [10], keratinocytes [11, 12], fibroblasts [13–16], osteoblasts number, and osteoblast function [17] as well as for human alveolar bone cells [18]. In contrast to that, the clinical use of CHX after oral surgical interventions, tooth extractions or implant placement showed significantly less signs of inflammation and improvement of gingival health by reduction of the microbial contamination of the wound [19–21]. A further disadvantage is given by the inactivation of CHX by binding on blood, serum proteins and sulcus fluid which was shown in in vitro studies [22, 23].

Thus, due to its possible cytotoxic effects, which has been shown in vitro for different cell types, as mentioned above [11–18], it is not recommended to irrigate bone or open wounds with CHX. Moreover, due to its inactivation through blood or serum [22, 23], the usefulness of subgingival CHX application is questionable.

In order to overcome these drawbacks, there is an ongoing search for alternative nonstaining, antibacterial irrigating solutions with comparable effects to CHX. Furthermore, there is a search for solutions which are also active in the subgingival environment, thus having a potential for disinfecting bone or at least for removing adherent bacteria from bone during periodontal, peri-implant or endodontic surgery.

Taurolidine is a derivative of the endogenous (sulfanyl) amino acid taurine. It has a broad spectrum of activity against gram-positive and gram-negative bacteria, anaerobic organisms and fungi and has been successfully used to prevent infections after abdominal surgery or as an adjuvant therapeutic agent against local and diffuse peritonitis (appendicitis perforate), acute and chronic osteitis, as well as against purulent, stercoral bacterial and of other genesis [24–27]. Its anti-adhesive properties are long known [28, 29] and were confirmed recently together with a better understanding of its mechanism of action [30]: Taurolidine is impacted by its easy hydrolysis in aqueous solution and is a flexible molecule that is capable of conformational adaptation to the requisite geometries needed for biological activity. Moreover, it has antiendotoxin properties and it reduces the adherence of bacteria to human epithelial cells. The authors in the cited study [30] see no evidence for interaction between taurolidine and peptidoglycan (of the cell wall) although former studies support this mechanism of action. They favour anti-adhesion properties against gram-negative bacteria by interaction with fimbriae proteins. For gram-positive bacteria and fungi, which do not display fimbriae proteins, they propose that hydrolysis products could induce reactivity and imply a more general mechanism of action. Moreover, recent studies have found antineoplastic activity and discuss its role in cancer treatment [31, 32].

Studies examining the effect of taurolidine on oral pathogens are rare. Its antibacterial efficacy on oral pathogens was tested using minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC)—however, only on 10 single species and not typical for dental biofilm [33]. In these studies MIC of taurolidine was only minimally higher than that of CHX (MIC of 0.12–0.5 mg/ml compared to MIC of 0.03–0.12 mg/ml, respectively).

Similar to CHX, taurolidine generally did not show any bacterial resistance due to this relatively unspecific pathway [34, 35]. Since it is used in abdominal surgery and on mucous membranes, it can be suggested that it can be irrigated on mucosal tissues without damage to soft tissues or bone.

Nevertheless, its effect on the oral flora has to be proven. In a clinical study with 16 subjects, taurolidine 2% could show similar effect like a biguanid (Vantocil 0.1%) on sulcus plaque index and a better effect on plaque index [36]. Reynolds et al. (1991) found significantly reduced bacterial surface growth in vitro and significantly reduced in vivo plaque regrowth (concerning plaque area, not plaque scores) by a 2% taurolidine solution, which was however, not as effective as a chlorhexidine rinse [37, 38]. These were so far the only clinical studies testing the effect in the oral cavity.

Thus, the aim of this study was to investigate the effect of a 2% taurolidine solution on the vitality of the established human supragingival plaque biofilm. Chlorhexidine (0.2%) and saline (NaCl) served as positive and negative control.

The hypothesis was that the *ex vivo* treatment of dental biofilm with taurolidine will significantly reduce bacterial vitality.

Material and methods

Study population

Eighteen healthy subjects (8 male, 10 female) with a mean age of 44.4±10.5 years were recruited. Exclusion criteria were the intake of antibiotics or other medicaments during the last 6 months, which could have influenced plaque accumulation, poor oral hygiene, crowns or restorations on the teeth evaluated; known allergy against mouth rinses; an age under 18 years and a pregnancy. Participants agreed and signed an informed consent prior to the start of the study.

Study procedure

At the beginning of the experiment, all participants were given a professional tooth cleaning. For the following 24 h,

they had to refrain from any kind of oral hygiene measures. At the next day, from each of the volunteers, a voluminous plaque biofilm sample was taken with a sterile probe (EXS 9; Hu-Friedy) from the vestibular surfaces of one upper and one lower first molar, streaked on a slide and divided into three equal parts. Each part was mounted with 5 μl of the following solutions using sterile pipette tips:

1. NaCl solution (Ringer 0.9%, negative control)
2. 2% Taurolidine solution (taurolidine, TauroSept®; Geistlich Pharma AG, Wollhusen, Switzerland)
3. 0.2% Chlorhexidine (positive control; Chlorhexamed forte®; GlaxoSmithKline, Bühl, Germany)

After a reaction time of 2 min, the test solutions were sucked off, and subsequently, the treated samples were vital stained according to the vital fluorescence technique as described in detail elsewhere ([39], modified in [7]). Briefly, the technique is based on the use of fluoresceindiacetate (FDA) and ethidium bromide (EB). FDA, a fluorescent dye, is not fluorescent but membrane soluble. In vital cells it is metabolised to fluorescein which is green and cannot leave the cell so that living cells are stained green. Dead cells are not able to metabolise the FDA so that there is no staining. A contra-staining with EB binds to the nucleic acids of dead cells and stains red.

After a staining reaction for 2 min, a cover glass was pressed firmly down onto the sample and the evaluation performed under a microscope (Axio Imager.Z2; Carl Zeiss, Göttingen). The samples were visually scanned and a script (AxioVision 4; Carl Zeiss, Göttingen) was used, which automatically scans the sample (from top left to down right) and stores four non-overlapping pictures using a digital camera (AxioCam MRm; Carl Zeiss, Göttingen). To ensure blindness, the products were numbered (CHX, 1; NaCl, 2; taurolidine, 3) and this number was used for coding the stored pictures (e.g. image 1_3_4: chlorhexidine, subject 3, fourth image). Finally, an image analysis software (AxioVision 4; Carl Zeiss) discriminating between green and red pixels was used to calculate the vitality of the bacterial biofilm flora, which means the percentage of vital bacteria in the total flora (VF%), averaging the data of the four pictures. Image analysis was performed by an investigator (TA), not otherwise involved in the study and unaware of the corresponding product, who listed the results under product number 1, 2 and 3.

Image analysis was performed predominantly objectively and both examiner and investigator of image analysis have an experience of more than 10 years with this kind of vitality analysis.

Statistical analysis

After decoding, mean values of VF% for each product were calculated using PASW Statistics (former SPSS) 18. Since analysis of variance (ANOVA) detected significant differences between the products and data showed normal distribution using the Kolmogorov–Smirnov test, Student's paired t test was used to detect differences between the products. For all analysis a difference was considered significant at the 95% confidence level (α=0.05).

Results

The results are summarized in Table 1. Plaque biofilm treated with the NaCl solution showed a mean vitality (VF%) of 82.42±6.04%. The plaque samples treated with CHX demonstrated VF of 34.41±14.79%, which was significantly lower than NaCl (p<0.001) and taurolidine (p<0.05). Taurolidine showed a VF of 47.57±16.60%, which was a statistically significant reduction (p<0.0001) compared to saline but not as pronounced compared to the positive control (p=0.017).

Figure 1 shows microscopic images of stained plaque biofilm samples after treatment with (a) NaCl, (b) CHX or (c) taurolidine.

Some taurolidine images of some volunteers (as shown in Fig. 1c) showed an interesting vitality pattern, which was never seen before in the working group with other antibacterial agents: completely red parts next to green parts. Possibly, taurolidine has a specific effect on specific oral bacteria, which confirms the need for further investigating this agent.

Discussion

The present study has evaluated the antibacterial effect of a 2% taurolidine solution on supragingival oral biofilm in comparison to saline (negative control) and a 0.2% chlorhexidine (positive control). The in vivo grown biofilm was carefully removed from the tooth surfaces and then tested

Table 1 Mean (±SD) of VF

	VF (n=18)	p value	% reduction
NaCl	82.42±6.04		
Taurolidine	47.57±16.60	<0.001[a]*	42.3[a]
		0.017[b]**	
CHX	34.41±14.79	<0.001[a]*	58.3[a]

Statistical comparison by ANOVA and paired t test

*p<0.001; **p<0.05

[a] Compared to NaCl

[b] Compared to CHX

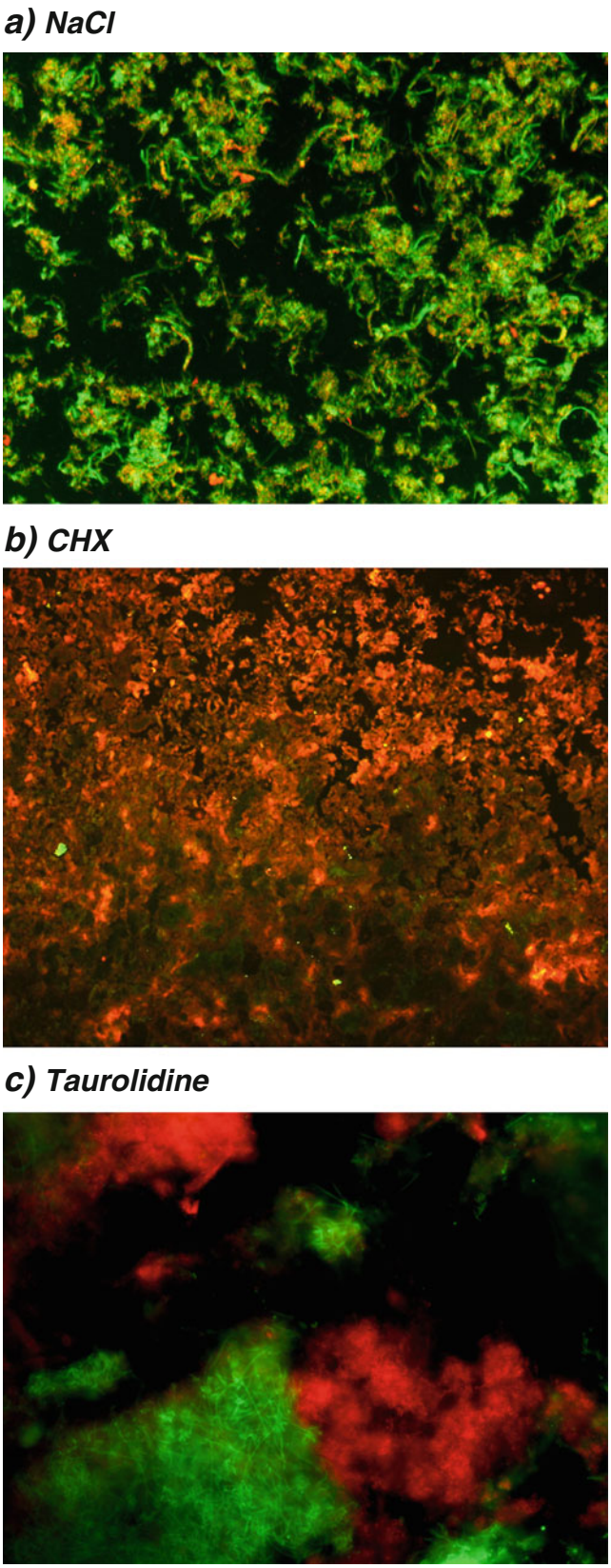

Fig. 1 **a**–**c** Representative microscopic images (×200 magnification) after treatment with **a** NaCl, **b** chlorhexidine or **c** taurolidine

(*ex vivo*). This laboratory study design is a first step in testing all properties of this relatively unknown substance in dentistry and—compared to cell suspensions and determining MICs—this model is much more close to the real situation. Thus, due to the fact that dental plaque exists as a biofilm, it was suggested that biofilm-based assays are of greater value than cell suspensions when assessing the effectiveness of chemical agents for the treatment and/or prevention of inflammatory periodontal disease [40]. Thus, some working groups use mixed (six) species in vitro biofilm models to imitate supragingival plaque; they cultivate and incubate the bacterial species to produce biofilms to be as close as possible to the intraoral situation [41]. While six microorganism only reflect a small part of the numerous bacterial flora with more than 800 species, the present study used an intraorally grown plaque biofilm, which represents the complex oral environment and has—however—to be processed within few minutes. The study design was based on a former study in which—for the first time—antibacterial properties of an enamel matrix protein (Emdogain®) against supragingival biofilm were detected [42] and were also confirmed later in a clinical experiment [43].

Taurolidine showed a significant effect compared to the control, but could not reach the strong antibacterial effect of chlorhexidine (58.3% reduction compared to NaCl). The mean relative reduction of 42%, however, indicates that the 2% taurolidine is a strong antibacterial substance.

The present reductions can be compared to a similar *ex vivo* study where—in comparison to NaCl—nearly the same reduction of CHX was found (57.8%), and the tested agent Emdogain® (EMD) showed a reduction of 28.9% [42]. The corresponding in vivo study revealed reductions of 19% for EMD and 35% for CHX also compared to NaCl [43]. Based on these results, where CHX and EMD had 60% and 30% reduction in vitro and then still significant reduction of 35% and 19% in vivo, it may be assumed that taurolidine will still have a significant antibacterial effect in the oral environment.

It should, however, be kept in mind that the present findings represent data from an *ex vivo* dental plaque-model. Therefore, further studies are warranted to definitively clarify the clinical effect of taurolidine on an in situ dental plaque biofilm, where different variables present in the mouth (inhibiting or promoting), e.g. dilution, washout, substantivity or anti-adhesive activity can be considered, and a possibly specific mode of action can be examined.

In conclusion, the present findings suggest that taurolidine possesses a strong antibacterial effect on the supragingival plaque biofilm thus warranting further evaluation in vivo.

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