ORIGINAL ARTICLE

Arch. Immunol. Ther. Exp., 2006, **54**, 61–68
PL ISSN 0004-069X

DOI 10.1007/s00005-006-0007-1

Susceptibility of *Propionibacterium acnes* and *Staphylococcus epidermidis* to killing by MPO-halide system products. Implication for taurine bromamine as a new candidate for topical therapy in treating acne vulgaris

Janusz Marcinkiewicz[1], Rafał Biedroń[1], Anna Białecka[2], Andrzej Kasprowicz[2], Monika Mak[1] and Marta Targosz[3]

[1] Department of Immunology, Jagiellonian University Medical College, Cracow, Poland
[2] Center of Microbiological Research and Autovaccines Ltd., Cracow, Poland
[3] Institute of Physics, Jagiellonian University, Cracow, Poland

Received: 2004.02.23, **Accepted:** 2005.08.10, **Published online first:** 2006.02.13

Abstract

Introduction: Taurine chloramine (TauCl) and taurine bromamine (TauBr) are the main haloamines produced by activated neutrophils. TauCl exerts both anti-inflammatory and microbicidal activities. Clinical studies showed that TauCl may be useful as an antimicrobial agent in the local treatment of infections. Much less is known about TauBr. Circumstantial evidence suggests that *Propionibacterium acnes* (PA) has a role in the inflammation of acne. Available topical therapies include antimicrobial agents which reduce total PA numbers and anti-inflammatory agents which suppress activity of the cells present in acne inflammatory lesions. In this study the bactericidal activities of TauBr and TauCl against PA and *Staphylococcus epidermidis* (SE), as a control strain, were investigated. Moreover, the influence of these haloamines on the generation of reactive oxygen species (ROS) by activated neutrophils was also tested.
Materials and Methods: TauBr and TauCl were prepared by reaction of taurine with HOBr and HOCl, respectively. The reaction was monitored by UV absorption spectra. The bactericidal activities of TauBr and TauCl were determined by the pour-plate method. The generation of ROS by neutrophils was determined by luminol chemiluminescence assay.
Results: In our experimental set-up, TauBr showed stronger antibacterial activity than TauCl. Interestingly, PA was significantly more susceptible to TauBr than SE was. Moreover, TauBr at non-cytotoxic concentrations significantly reduced ROS generation by neutrophils.
Conclusions: Since PA is considered to be an etiological agent in acne and ROS are closely correlated with the pathogenesis of inflammatory skin diseases, the reported data suggest that TauBr may be a good candidate for the topical therapy for acne vulgaris.

Key words: acne vulgaris, *Propionibacterium acnes, Staphyloccocus epidermidis*, taurine bromamine, N-bromotaurine, taurine chloramine, N-chlorotaurine, bactericidal activity, anti-inflammatory properties.

Abbreviations: PA – *Propionibacterium acnes*, SE – *Staphyloccocus epidermidis*, HOCl – hypochlorous acid, TauBr – taurine bromamine (N-bromotaurine), TauCl – taurine chloramines (N-chlorotaurine), ROS – reactive oxygen species, PMNs – neutrophils, H_2O_2 – hydrogen peroxide, HOBr – hypobromous acid, MPO – myeloperoxidase, PBS – phosphate-buffered saline, AFM – atomic force microscopic, HBSS – Hank's balanced salt solution, LCL – luminol-dependent chemiluminescence, c.f.u. – colony forming unit, cps – count per second.

Corresponding author: Janusz Marcinkiewicz, Department of Immunology, Jagiellonian University Medical College, Czysta 18, 31-121 Kraków, Poland, tel./fax: +48 12 633-94-31, e-mail: mmmarcin@cyf-kr.edu.pl

INTRODUCTION

Acne vulgaris is the most common skin disorder and its etiology appears to be multifactorial [5]. The four main etiological factors are hypercornification of the pilosebaceus duct, increased sebum production, colonization with skin bacteria and, subsequently, the production of inflammation [1]. Acne lesions are mainly colonized by

two coexisting groups of bacteria: staphylococci (*Staphylococcus epidermidis* (SE)) and anaerobic diphtheroids (*Propionibacterium acnes* (PA) and *Propionibacterium granulosum*) [14]. If microbial flora is significant in the pathogenesis of acne, the most likely organism to blame is PA [5]. PA contributes to the inflammatory nature of acne by inducing phagocytes to secrete pro-inflammatory mediators. PA-induced chemotactic factors play an important role in attracting neutrophils (PMNs) to the pilosebaceus unit. PMNs subsequently phagocytize the bacteria, during which process lysosomal enzymes are released, possibly enhancing damage to the follicular epithelium. Moreover, reactive oxygen species (ROS) generated by activated PMNs have been reported to be capable of causing tissue injury, called auto-oxidative damage, at the sites of acne inflammation [1, 2]. Thus, acne therapy must address the etiological factors involved in the pathogenesis of acne and should result in attenuation of the inflammatory response.

A variety of immune cells are involved in the development of acne lesions. In the very early stages, $CD4^+$ T cells and macrophages appear around pilosebaceous follicles as a result of delayed type hypersensitivity response to a persistent antigen present within the ducts of pilosebaceous follicles [11]. On the other hand, the majority of cells involved in the late acne inflammatory lesions are PMNs [2]. Activated PMNs use hydrogen peroxide (H_2O_2) and halides (Cl^-, Br^-) to generate hypohalous acids (HOCl, HOBr) [3, 29, 30]. HOCl is considered the major bactericidal agent of PMNs *in vivo,* while hypobromous acid (HOBr) is primarily generated by eosinophils [12]. It was recently reported that HOBr may also be generated by the myeloperoxidase (MPO) system of PMNs [8]. Moreover, it has been shown that even physiological concentrations of bromide (1000-fold lower than that of Cl^-) enhance the bactericidal effects of MPO *in vitro* [10].

$$HOCl + Br^- \rightarrow HOBr + Cl^-$$

HOCl and HOBr are unstable, highly reactive toxic oxidants. They are potent microbicidal agents, but excessive or misplaced production can cause tissue damage [9, 13]. This deleterious effect is neutralized *in vivo* by the reaction of HOCl and HOBr with β-amino acids, which results in the production of more stable and less toxic haloamines [6, 31]. As taurine is the most abundant free amino acid in the leukocyte cytosol, the major haloamines are taurine chloramine (N-chlorotaurine, TauCl) and taurine bromamine (N-bromotaurine, TauBr) [15, 33]. TauCl formation protects cells from damage by HOCl generated at a site of inflammation. Moreover, *in vitro* studies showed that TauCl has both microbicidal and anti-inflammatory properties [18, 20, 24, 28]. TauCl used at micromolar concentrations inhibits *in vitro* the production of many inflammatory mediators, such as tumor necrosis factor α, PGE_2, nitric oxide, and interleukin 8 [18, 20, 28], while a microbicidal activity of TauCl has been demonstrated at physiological micromolar and supraphysiological millimolar concentrations [23, 24]. Nevertheless, TauCl has been conceived to be useful as an antimicrobial agent in the treatment of local infections [25, 26].

In contrast to TauCl, much less is known about the biological properties of TauBr. For instance it has been shown that both TauBr and TauCl are able to kill the schistosomula of *Schistosoma mansoni* [35]. Only our recent studies indicate that TauBr may play a role in inflammation. We have shown that TauBr exerts even stronger bactericidal properties than TauCl [21]. Moreover, both TauBr and TauCl induce expression of heme oxygenase-1 (HO-1), a stress-induced enzyme with strong anti-inflammatory properties [27]. If TauBr and TauCl show both anti-microbial and anti-inflammatory properties, it is tempting to speculate that they can be used as a disinfectant for topical use in humans, for example in acne vulgaris.

To verify this hypothesis we tested the *in vitro* microbicidal activities of TauBr and TauCl against PA isolated from acne inflammatory lesions. SE, a common bacterium of the skin microflora which is not involved in the pathogenesis of acne vulgaris, was used as a control bacterial strain. Moreover, we addressed the issue whether taurine haloamines can inhibit ROS generation by PMNs, as they are supposed to be closely correlated with the pathogenesis of a variety of inflammatory skin diseases.

MATERIALS AND METHODS

Synthesis and determination of TauCl and TauB

TauCl was prepared by drop-wise addition of 5 ml of a 20 mM NaOCl (Aldrich, Germany) solution in 0.05 M phosphate buffer (pH 7.4) under vigorous stirring to 5 ml of 24 mM taurine (Sigma, Germany). Each preparation of TauCl was monitored by UV absorption spectra (λ=200–400 nm) to assure the authenticity of monochloramine (TauCl) (λ_{max}=252 nm) and the absence of dichloramine ($TauCl_2$) (λ_{max}=300 nm) and unreacted HOCl/OCl (λ_{max}=292 nm). The concentration of synthesized TauCl was determined using the molar extinction coefficient of 429 $M^{-1}cm^{-1}$ at A_{252} [31]. In the preparation of TauBr, HOBr/OBr^- was first generated by mixing equimolar amounts of $HOCl^-$ and $NaBr^-$. Then the product of this reaction was mixed with taurine. The mono-bromamine was obtained with a 10-fold excess of amine over the amount of HOBr/OBr^-. Each preparation of TauBr was monitored by UV absorption spectra (λ=200–400 nm) to assure the authenticity of TauBr (λ_{max}=288 nm). The concentration of synthesized TauBr was determined using the molar extinction coefficient of 415 M^{-1} cm^{-1} at A_{288} [30]. Stock solutions of TauCl and TauBr were kept at 4°C for a maximum of 7 days before use.

Bacterial strains

PA was isolated from acne lesions of patients with mild acne vulgaris. For the present study we selected PA

No. 496, a strain which belongs to biotype 1 (sorbitol$^+$, erythritol$^+$, ribose$^+$), the most common biotype of *Propionibacterium* isolated from acne patients [4]. SE was isolated from normal human skin. The selected micro-organisms were lyophilized and stored until used. To prepare a culture of the given strain for the investigations, the lyophilized material was revived and grown in the following media: PA in Schaedler Agar Base (Difco, USA) at 35°C for 72 h in anaerobic conditions and SE in Tryptic Soy Broth (Difco, USA) at 35°C for 18 h in aerobic conditions. Bacteria were centrifuged at 1800×g, washed twice with 0.9% NaCl, and diluted in saline to a concentration of 1×10^8 c.f.u./ml before use.

Bactericidal activity of TauBr, TauCl, and HOCl

Briefly, bacteria were suspended and further diluted in either phosphate buffer saline (PBS; pH 7.4) or sodium acetate buffer (pH 5.0) to achieve a final concentration of 1×10^5 c.f.u./ml and then were incubated with different concentrations of the components (1–1000 μM). Immediately after the incubation (30 min.), aliquots were removed and the viable cell count was determined by the pour-plate method [21, 24]. Control samples (bacteria diluted in the buffers only) were treated the same way. The bactericidal activities of TauBr, TauCl, and HOCl are expressed as log c.f.u./ml of bacteria survived. The detection limit was 5 c.f.u./ml.

Atomic force microscopic imaging of PA

Atomic force microscopic (AFM) imaging was performed by sensitive monitoring of the atomic force interaction between the cantilever tip of the microscope and an object during the scanning movement of the tip over the object surface. Such a procedure requires the object to be immobilized while the scanning is performed, which is particularly important for biological samples.

In our experimental set-up, bacteria were killed in different ways: by heat (60°C for 2 h), TauBr treatment (50 μM, 30 min) or antibiotic treatment (clindamycin, 15 μg/ml, 24 h). Live bacteria were used as a control. The tested bacteria were tightly bound to the surface of commercially available silanized glass slides. The positively charged surface of such substrates promoted irreversible adhesion of the investigated bacteria subjected to the following treatment: the slide was coated with a bacterial suspension and left aside for 1 h, then it was flushed with 2.5% glutaraldehyde. After 5 min the slide was rinsed carefully with PBS and stored for imaging on the same day. It was found that the addition of glutaraldehyde does not change the dimensions and shape of the bacteria. The AFM experiments were performed with a Thermomicroscopes CP AFM/STM microscope equipped with a 100 μm scanner and a commercial liquid cell (Thermomicroscopes Microcell). In this system the cantilever remains stationary, whereas the sample is moved by the piezoelectric tube. Silicon nitride (Si_3N_4) cantilevers (Park Scientific Instruments unsharpened Ultralevers) with a spring constant of 0.1 N/m and conical-shape tips were used for contact-mode imaging. Typically, images of 256×256 pixels were collected with a force applied to the cantilever of 2 nN, and the scan rate was maintained below 0.7 line/s.

Mice

Inbred BALB/c female mice (8–12 weeks of age, 18–22 g) were maintained at the Animal Breeding Unit, Department of Immunology, Jagiellonian University Medical College, Cracow. All mice were housed 4–5 per cage in the laboratory room with water and standard diet *ad libitum*. The appropriate permission for using mice in this project was obtained from the Local Ethics Committee.

Cells

Peritoneal mouse PMNs were elicited by intraperitoneal injection of 2 ml Markol 52 oil (Exon, USA). Cells were collected 16 h later by washing out the peritoneal cavity with 5 ml of PBS containing 5 U heparin/ml (Polfa, Poland). Cells were centrifuged and red blood cells were lysed by osmotic shock using distilled water. Osmolarity was restored by adding 2× concentrated PBS. The cells collected were at least 90–95% PMNs, as assessed by microscopic examination of cytocentrifuge preparations fixed in methanol and stained with May Grunwald/Giemsa (Merck, USA). The presence of macrophages (3–7%) was judged by cytochemical demonstration of non-specific esterase-positive mononuclear cells using α-naphtyl acetate [34].

Luminol chemiluminescence assay

Chemiluminescence was counted at 37°C in a temperature-stabilized Lucy 1 luminometer (Anthos, Austria). PMNs (1×10^7 cells/ml) in Hank's balanced salt solution (HBSS) were incubated in vials with luminol sodium salt (0.4 mg/ml; Sigma, Germany). After a darkness adaptation period of 15 min at 37°C, the cells (100 μl/well) were quickly transferred to a 96-well flat-bottom black plate (Nunc, Denmark) containing 100 μl of PA (3×10^8 c.f.u./ml) in HBSS, and photon emission over 75 min with 3-min intervals was measured. In other experiments, PMNs were placed in a black plate with luminol for 20 min at 37°C. After darkness adaptation, TauBr or clindamycin, the potential inhibitors of chemiluminescence, were added and the cells were stimulated with opsonized zymosan (0.4 mg/ml; Sigma, Germany). Each type of experiment was performed in duplicate.

Statistics

The statistical significance of differences between groups was analyzed using a factorial ANOVA (Microsoft Excel) followed by the Student's *t*-test, if appropriate. A p-value less than 0.05 was considered statistically significant.

RESULTS

Susceptibility of PA and SE to TauBr, HOCl, and TauCl at 37°C

In our experimental set-up, the bactericidal activity of TauBr, TauCl, and HOCl, at concentrations ranging 1–1000 μM, was tested at pH 7.4 and pH 5.0 and at two different temperatures, 37°C and 23°C. To exclude the influence of acidity on bacterial survival, PA and SE were incubated either in PBS only (pH 7.4) or in sodium acetate buffer only (pH 5.0). At these pH values (5.0 and 7.4) we did not observe any differences in the survival of bacteria. In these conditions, full stability of TauBr and TauCl was observed during the short incubation time (30 min).

Micromolar concentrations of all the tested agents demonstrated considerable bactericidal activity against PA and SE when incubated at 37°C (Fig. 1). At this temperature, relevant to the conditions at a site of inflammation, HOCl killed both bacteria tested equally effectively. The bactericidal activity of TauBr was similar to that of HOCl. Surprisingly, under these conditions, HOCl and TauBr exerted stronger bactericidal activity at pH 7.4 than at pH 5.0. TauCl bactericidal activity was different from those of HOCl and TauBr. At neutral pH, TauCl did not kill SE at the concentrations used (<1000 μM) and showed weak bactericidal activity against PA after incubation for 30 min. Moreover, the bactericidal activity of TauCl against both tested bacteria was increased by an acidic pH and differences in the susceptibilities of PA and SE to TauCl were observed (Fig. 1).

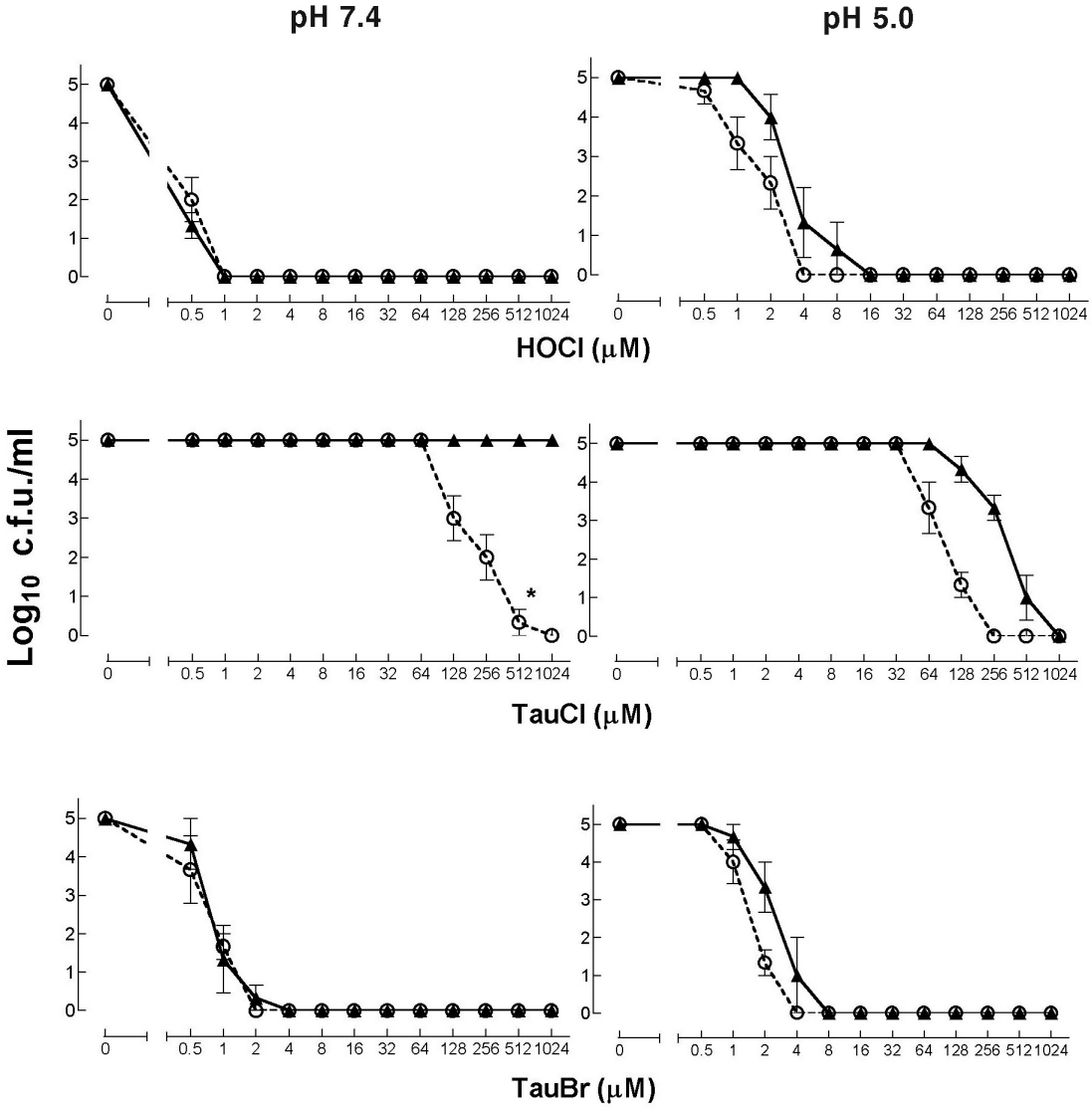

Fig. 1. Susceptibility of PA (○) and SE (▲) to TauBr, TauCl, and HOCl at 37°C. The reaction mixture contained 1×10^5 c.f.u./ml and TauCl, TauBr, or HOCl at the concentrations indicated (μM). Samples were incubated either in phosphate buffer, pH 7.4, or in sodium acetate buffer, pH 5.0, for 30 min. The reduction of bacteria growth was estimated as described in Materials and Methods. Each point of the killing curves represents the mean (±SE) calculated from 3 separate experiments. The asterisks indicate a significant ($p<0.05$) difference in bactericidal activity against PA and SE.

Susceptibility of PA and SE to TauBr, HOCl and TauCl at 23°C

In further experiments, the bactericidal activity of the agents was tested by treatment of bacteria at 23°C, since in a lower reaction temperature (23°C vs. 37°C) the differences between HOCl, TauBr and TauCl in their bactericidal activity were more pronounced (Fig. 2).

At this temperature, acidification enhanced the bactericidal activity of HOCl. Moreover, HOCl, at acidic pH, was more effective against PA than SE (Fig. 2). The bactericidal activity of TauCl was significantly weaker than that of HOCl, and under these experimental conditions TauCl was active only at pH 5.0 (the effect of dichloramine). Importantly, PA was more sensitive than SE to TauCl. TauBr, as well as HOCl and TauCl, showed statistically significant higher activity at acidic pH than at neutral pH. TauBr exerted stronger bactericidal activity than TauCl to all test bacteria. Most importantly, the study revealed significant differences in the susceptibility of PA and SE to TauBr at 23°C (Fig. 2).

AFM images of PA treated with TauBr

Fig. 3 demonstrates changes in PA morphology killed with TauBr, clindamycin, or heat-shock by means of AFM. The samples treated with TauBr (50 μM) and clindamycin (15 μg/ml) showed similar changes in the morphology of the bacterial surface and cytosol. The destruction of bacterial cell components was much stronger than that observed in heat-killed bacteria. Further studies are necessary to characterize the mor-

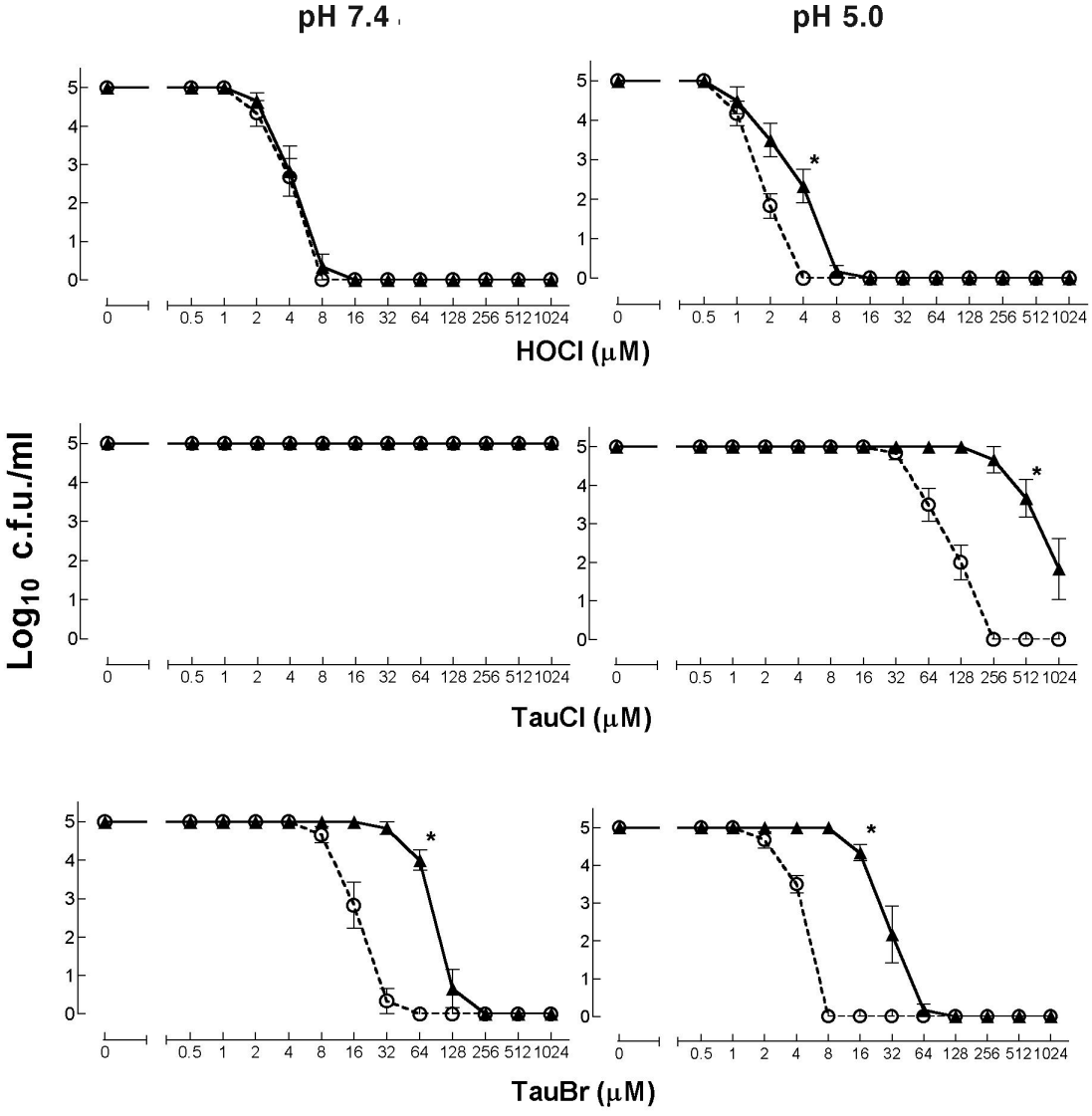

Fig. 2. Susceptibility of PA (○) and SE (▲) to TauBr, TauCl, and HOCl at 23°C. The reaction mixture contained 1×10^5 c.f.u./ml and TauCl, TauBr, or HOCl at the concentrations indicated (μM). Samples were incubated either in phosphate buffer, pH 7.4, or in sodium acetate buffer, pH 5.0, for 30 min. The reduction of bacteria growth was estimated as described in Materials and Methods. Each point of the killing curves represents the mean (±SE) calculated from 9 separate experiments. The asterisks indicate a significant ($p<0.05$) difference in bactericidal activity against PA and SE.

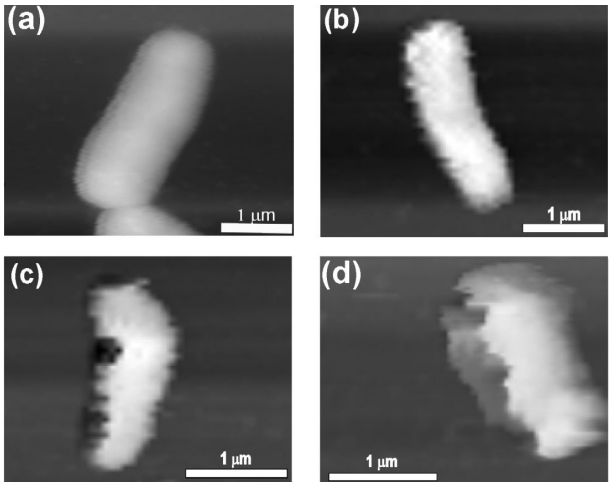

Fig. 3. AFM images of PA. (**a**) Untreated control bacteria; (**b**) heat-killed bacteria; (**c**) clindamycin-killed bacteria; (**d**) TauBr-killed bacteria. For details see Materials and Methods.

phological effect of killing concentrations of TauBr on target bacteria. The addition of glutaraldehyde (a control – see Materials and Methods) did not change the dimensions and shape of the bacteria.

ROS generation by PMNs incubated with TauBr and TauBr-killed bacteria

The generation of ROS by activated PMNs was measured using luminol-dependent chemiluminescence (LCL). Upon addition of zymosan, neutrophil LCL increased light emission approximately 20 times over baseline, reaching a maximum about 8 min after application of the stimulus. When TauBr was added to the reaction mixture, a dose-dependent decrease in LCL was observed. At a concentration of 300 μM, TauBr completely suppressed LCL (Fig. 4). By contrast, clindamycin up to a concentration of 50 μg/ml did not affect LCL. In other experiments the ability of PA to stimulate ROS generation was determined. Bacteria were killed either by TauBr, clindamycin, or heat-shock. No significant differences in LCL were observed among the groups (Fig. 5).

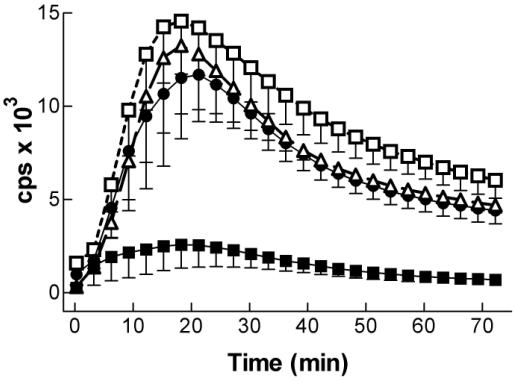

Fig. 5. LCL of PMNs (1×10^6 per well) stimulated with PA killed by: heat-shock (△), TauBr (□), or clindamycin (●). Unstimulated control cells (■). Bacteria/PMN ratio was 30:1. Each point represents the mean (±SE) of 3 experiments.

DISCUSSION

The aim of this study was to determine whether TauCl and/or TauBr, due to their recently described bactericidal and anti-inflammatory properties [21, 27], may be taken into consideration for topical treatment of acne vulgaris. Available topical therapies for acne include comedolytic, bactericidal, and anti-inflammatory agents. Thus, acne therapy must address the etiological factors involved in acne pathogenesis (PA) and should result in diminishing the tissue damage by inflammatory mediators. Indeed, PA appears to be the target for oral and topical application of antimicrobials [16, 36]. Moreover, the antibiotics used for the treatment of acne significantly inhibit ROS generated by PMNs when compared with other antibiotics [1, 2, 32].

In this study we tested the susceptibility of PA and SE to MPO-halide system products used at micromolar concentrations. Our data confirm that HOCl at physiological concentrations exerts strong bactericidal activity, which clearly supports its contribution in the neutrophil defense system [13, 19]. However, due to its very high toxicity and extreme instability, HOCl is not used clinically as an antimicrobial agent, even in a local treatment. On the other hand, TauCl, a less toxic and more stable antimicrobial agent of stimulated PMNs, has been conceived to be useful as a disinfectant in human

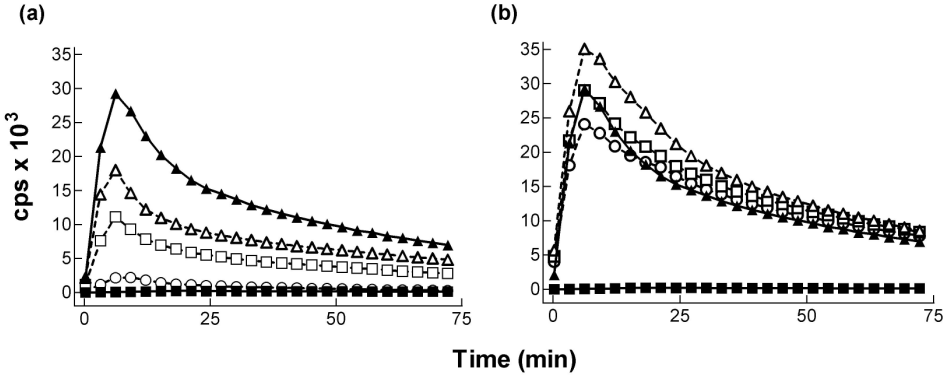

Fig. 4. Influence of TauBr and clindamycin on LCL. PMNs (1×10^6 per well) were stimulated with opsonized zymosan (0.4 mg/ml) in the presence of: (**a**) TauBr 50 (△), 100 (□), 300 (○) μM, and (**b**) clindamycin 5 (△), 15 (□), 45 (○) μg/ml. Controls: zymosan stimulated cells (▲); unstimulated cells (■). The figure shows the results of one of 3 independent experiments.

medicine [23, 24]. Recent clinical studies showed that the topical application of TauCl to body sites could be considered as a therapeutic option [25, 26]. To achieve significant killing of pathogens by a 1% solution of TauCl (55mM), incubation times ranging from 10–60 min were needed [22]. It has been also reported that TauCl used at micromolar concentrations exerted bactericidal activity, but killing times extended to a few hours [24]. In our experimental set-up, TauCl incubated with PA or SE for 30 min hardly affected bacterial growth. As long exposure times are difficult to achieve when disinfectants are used topically, TauCl at micromolar concentrations cannot be used in the treatment of acne vulgaris. In this study, the bactericidal activities of TauBr at low, non-cytotoxic concentrations have been demonstrated for the first time. Our data clearly indicate that TauBr exerts *in vitro* much stronger bactericidal activity than TauCl. TauBr's effectiveness in bacteria killing was close to that of HOCl, the most potent bactericidal agent of the MPO-halide system [9]. Importantly, tremendous differences in bactericidal potency between TauBr and TauCl were shown at neutral pH. Under these conditions, TauCl (up to 1 mM) did not affect bacteria growth at all. From a clinical point of view, it is very interesting that the susceptibility of PA to TauBr appeared to be significantly higher than that of SE. Both species belong to the bacterial flora of the skin, but only PA is considered to be involved in the pathogenesis of the chronic skin inflammation in acne vulgaris [5, 11, 14]. Therefore, TauBr, due to its ability to selectively kill PA when used at micromolar concentrations, seems to be a better candidate than TauCl as a topical disinfectant agent in acne.

During the last few years, benzoyl peroxide and clindamycin have been the two most widely prescribed topical drugs in the treatment of acne [7, 17]. Benzoyl peroxide shows antibacterial activity and decreases inflammatory damage by inhibiting the release of ROS through the killing of PMNs. Clindamycin, a bacteriostatic/bactericidal antibiotic, suppresses the complement-derived chemotaxis of PMNs, thereby reducing the potential for inflammation [32]. To determine whether TauBr, similarly to clindamycin and benzoyl peroxide, will also affect the function of PMNs in acne inflammatory lesions, we asked the question whether TauBr used at microbicidal concentrations for PA but lower than the minimal bactericidal concentration for SE affects ROS generation by PMNs. Our data indicate that PA bacteria killed by TauBr retain their ability to stimulate the generation of ROS by PMNs. This may suggest that phagocytosis of TauBr-treated bacteria is not affected despite the damage to the bacteria cell wall surfaces. On the other hand, the direct effect of TauBr on PMNs results in the inhibition of ROS generation by the cells. Alternatively, the inhibition of LCL by TauBr may be explained by its ability to react with H_2O_2, the major ROS of PMNs [13, 21]. These properties of TauBr seem to be crucial for anti-acne therapy, since recent studies have highlighted the importance of ROS in mediating acne inflammation. For instance, clindamycin and benzoyl peroxide, the two most commonly prescribed topical antimicrobials in the treatment of acne vulgaris, have demonstrated anti-inflammatory properties by suppressing the inflammatory damage caused by PMNs [17, 32]. Moreover, it has been shown that patients with papulo-pustular type acne showed a significantly increased level of hydrogen peroxide produced by PMNs compared with healthy controls [1, 2].

In conclusion, the data presented above indicate that TauBr at micromolar, non-cytotoxic concentrations exerts bactericidal activity *in vitro* which is significantly stronger than that of TauCl. The fact that PA, an etiological factor of acne, is more susceptible to TauBr than SE is supports the concept of using TauBr as a selective topical disinfectant in the treatment of acne vulgaris. Moreover, TauBr showed the capacity to reduce the generation of ROS by PMNs, which seems to be crucial for reducing the number and severity of inflammatory lesions in patients with mild to moderately severe acne vulgaris.

REFERENCES

1. Akamatsu H. and Horio T. (1998): The possible role of reactive oxygen species generated by neutrophils in mediating acne inflammation. Dermatology, **196**, 82–85.
2. Akamatsu H., Horio T. and Hattori K. (2003): Increased hydrogen peroxide generation by neutrophils from patients with acne inflammation. Int. J. Dermatol., **42**, 366–369.
3. Babior B. M. (1978): Oxygen dependent microbial killing by phagocytes. Engl. J. Med., **298**, 659–668.
4. Białecka A., Mak M., Biedroń R., Bobek M., Kasprowicz A. and Marcinkiewicz J. (2005): Different pro-inflammatory and immunogenic potentials of *Propionibacterium acnes* and *Staphylococcus epidermidis*: implication for chronic inflammatory acne. Arch. Immunol. Ther. Exp., **53**, 79–85.
5. Burkhart C. G., Burkhart C. N. and Lehmann P. F. (1999): Acne: a review of immunologic and microbiologic factors. Postgrad. Med. J., **75**, 328–331.
6. Carr A. C., van den Berg J. J. and Winterbourn C. C. (1998): Differential reactivities of hypochlorous and hypobromous acids with purified *Escherichia coli* phospholipids: formation of haloamines and halohydrins. Biochim. Biophys. Acta, **15**, 254–264.
7. Dreno B. (2004): Topical antibacterial therapy for acne vulgaris. Drugs, **64**, 2389–2397.
8. Gaut J. P., Yeh G. C., Tran, H. D., Byun J., Henderson J. P., Richter G. M., Brennan M. L., Lusis A. J., Belaaouaj A., Hotchkiss R. S. and Heinecke J. W. (2001): Neutrophils employ the myeloperoxidase system to generate antimicrobial brominating and chlorinating oxidants during sepsis. Proc. Natl. Acad. Sci. USA, **98**, 11961–11966.
9. Hampton M. B., Kettle A. J. and Winterbourn C. C. (1998): Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. Blood, **92**, 3007–3017.
10. Henderson J. P., Byun J., Williams M. V., Mueller D. M., McCormick M. L. and Heinecke J. W. (2001): Production of brominating intermediates by myeloperoxidase. A transhalogenation pathway for generating multagenic nucle-

obases during inflammation. J. Biol. Chem., **276**, 7867–7875.
11. Jeremy A. H., Holland D. B., Roberts S. G., Thomson K. F. and Cunliffe W. J. (2003): Inflammatory events are involved in acne lesion initiation. J. Invest. Dermatol., **121**, 20–27.
12. Jong E. C., Henderson W. R. and Klebanoff S. J. (1980): Bactericidal activity of eosinophil peroxidase. J. Immunol., **124**, 1378–1382.
13. Klebanoff S. and Hamon C. B. (1992): Role of myeloperoxidase mediated antimicrobial system in intact leukocytes. J. Reticuloendthel. Soc., **12**, 170–175.
14. Koreck A., Pivarcsi A., Dobozy A. and Kemeny L. (2003) The role of innate immunity in the pathogenesis of acne. Dermatology, **206**, 96–105.
15. Learn D. B., Fried V. A. and Thomas E. L. (1990): Taurine and hypotaurine content of human leukocytes. J. Leukoc. Biol., **48**, 174–182.
16. Leyden J. J. (2004): Antibiotic resistance in the topical treatment of acne vulgaris. Cutis, **73**, (suppl. 6), 6–10.
17. Leyden J., Kaidbey K. and Levy S. F. (2001): The combination formulation of clindamycin 1 % plus benzoyl peroxide 5 % versus 3 different formulations of topical clindamycin alone in the reduction of *Propionibacterium acnes*. An *in vivo* comparative study. Am. J. Clin. Dermatol., **2**, 263–266.
18. Marcinkiewicz J. (1997): Neutrophil chloramines – missing links between innate and acquired immunity. Immunol. Today, **18**, 577–580.
19. Marcinkiewicz J., Chain B., Nowak B., Grabowska A., Bryniarski K. and Baran J. (2000): Antimicrobial and cytotoxic activity of hypochlorous acid: interactions with taurine and nitrite. Inflamm. Res., **49**, 280–289.
20. Marcinkiewicz J., Grabowska A., Bereta J., Bryniarski K. and Nowak B. (1998): Taurine chloramine down-regulates the generation of murine neutrophil inflammatory mediators. Immunopharmacology, **40**, 27–38.
21. Marcinkiewicz J., Mak M., Bobek M., Biedroń R., Białecka A., Koprowski M., Kontny E. and Maśliński W. (2005): Is there a role of taurine bromamine in inflammation? Interactive effects with nitrate and hydrogen peroxide. Inflamm. Res., **54**, 42–49.
22. Nagl M. and Gottardi W. (1996): Enhancement of the bactericidal efficacy of N-chlorotaurine by inflammation samples and selected N-H compounds. Hyg. Med., **21**, 597–605.
23. Nagl M., Gruber A., Fuchs A., Lell C.P., Lemberger E.M., Borg-Von Zepelin M. and Wurzner R. (2002) Impact of N--chlorotaurine on viability and production of secreted aspartyl proteinases of *Candida* spp. Antimicrob. Agents Chemother., **46**, 1996–1999.
24. Nagl M., Hess M.W., Pfaller K., Hengster P. and Gottardi W. (2000): Bactericidal activity of micromolar N-chlorotaurine: evidence for its antimicrobial function in the human defense system. Antimicrob. Agents Chemother., **44**, 2507–2513.
25. Nagl M., Nguyen V. A., Gottardi W., Ulmer H. and Hopfl R. (2003): Tolerability and efficacy of N-chlorotaurine in comparison with chloramine T for the treatment of chronic leg ulcers with a purulent coating: a randomized phase II study. Br. J. Dermatol., **149**, 590–597.
26. Neher A., Nagl M., Appenroth E., Gstottner M., Wischatta M., Reisigl F., Schindler M., Ulmer H. and Stephan K. (2004): Acute otitis externa: efficacy and tolerability of N--chlorotaurine, a novel endogenous antiseptic agent. Laryngoscope, **114**, 850–854.
27. Olszanecki R. and Marcinkiewicz J. (2004): Taurine chloramines and Taurine bromamine induce heme-oxygenase-1 in resting and LPS-stimulated J774.2 macrophages. Amino Acids, **27**, 29–35.
28. Park E., Schuller-Levis G., Jia J. H. and Quinn M. R. (1997): Preactivation exposure of RAW 264.7 cells to taurine chloramine attenuates subsequent production of nitric oxide and expression of iNOS mRNA. J. Leukoc. Biol., **61**, 161–166.
29. Thomas E. L. (1979): Myeloperoxidase, hydrogen peroxide, chloride antimicrobial system: effect of exogenous system on antibacterial action against *Escherichia coli*. Infect. Immun., **25**, 110–116.
30. Thomas E. L., Bozeman P. M., Jefferson M. M. and King C. C. (1995): Oxidation of bromide by the human leukocyte enzymes myeloperoxidase and eosinophil peroxidase. Formation of bromamines. J. Biol. Chem., **270**, 2906–2913.
31. Thomas E. L., Grisham M. B. and Jefferson M. M. (1986): Preparation and characterization of chloramines. Methods Enzymol., **132**, 569–585.
32. Warner G. T and Plosker G. L. (2002): Clindamycin/benzoyl peroxide gel: a review of its use in the management of acne. Am. J. Clin. Dermatol., **3**, 349–360.
33. Weiss S. J., Klein R., Slivka A. and Wei M. (1982): Chlorination of taurine by human neutrophils. Evidence for hypochlorous acid generation. J. Clin. Invest., **70**, 598–607.
34. Yam L. T., Li C. T. and Crosby W. H. (1971): Cytochemical identification of monocytes and granulocytes. Am. J. Clin. Pathol., **55**, 283–290.
35. Yazdanbakhsh M., Eckmann C. M. and Roos D. (1987): Killing of schistosomula by taurine chloramine and taurine bromamine. Am. J. Trop. Med. Hyg., **37**, 106–110.
36. Zouboulis C. C. and Piquero-Martin J. (2003): Update and future of systemic acne treatment. Dermatology, **206**, 37–53.