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Albumin reduces the antibacterial activity of polyhexanide-biguanide-based antiseptics against *Staphylococcus aureus* and MRSA

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ARTICLE INFO

Article history:
Accepted 5 March 2013

Keywords:
Polyhexanide-biguanide
Antiseptics
Staphylococcus aureus
MRSA
Albumin
Antibacterial activity

ABSTRACT

Background: Wound infection is one of the major complications in acute and chronic wound healing. Antiseptic solutions and wound irrigating agents are routinely used for therapy and prevention in healthcare today. Even if wound exudate contains total protein concentrations up to 9.3% and albumin concentrations up to 2.7% its influence to the antibacterial efficacy of these agents is barely investigated.

Materials and methods: This study analyzed the antibacterial effect of polyhexanide biguanide (PHMB) agents (PHMB-concentration 0.005–0.1%) against *Staphylococcus aureus* and methicillin-resistant-*S. aureus* (MRSA) after 2 min incubation in presents of albumin in different concentrations (0–3%) in a standardized quantitative suspension assay.

Results: A significant decrease of the antibacterial activity against *S. aureus* was shown for a PHMB-concentration of 0.005% from 0.3% albumin ($p < 0.05$), respectively highly significant from 0.75% ($p < 0.01$) on. Thereby the loss of antimicrobial effect was presented as a linear correlation to the rising concentration of albumin. Furthermore a reduction of the antibacterial activity against MRSA in comparison to *S. aureus* was presented, for albumin concentrations from 3% on highly significant ($p < 0.01$).

Conclusion: The study showed that albumin causes a significant decrease of the antibacterial potency of PHMB-based antiseptics. Furthermore a diminished potency of the investigated substances for MRSA-contaminated wounds must be taken in consideration. If in vitro experiments show a significant decrease of antibacterial efficacy in the presence of albumin a sufficient activity of PHMB-based agents in clinical practice, especially in cases of exuding wounds or dried-up exudates, cannot be expected.

1. Introduction

Chronic wounds and wound infections cause a multidisciplinary challenge in daily healthcare. Wounds present an easy access for bacteria to human tissue that allow them to cause local infections that may result in systemic processes and septic organ failure [1,2]. More than 80% of leg ulcers are colonized by bacteria [3,4]. Furthermore bacterial colonization is closely associated with impaired healing. *Staphylococcus*

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0305-4179/$36.00
http://dx.doi.org/10.1016/j.burns.2013.03.003

aureus (*S. aureus*) was shown to be the most common pathogenic agent in clinical isolate from wounds representing 70% of isolates [5]. At present methicillin-resistant *S. aureus* (MRSA) have become endemic in some hospitals indicating the increasing importance of adequate treatment [6].

Antiseptic agents are routinely used in healthcare today and can be considered to be the first-line therapy for treatment and prevention of wound infections [7]. The philosophy behind local delivery of skin antiseptics is to raise tissue levels of antimicrobials to a level where sensitive and relatively insensitive organisms are inhibited and to avoid potential systemic side effects of high-dose antibiotics [4]. Several studies concluded that polyhexanide biguanide (PHMB) solutions are among the most suitable agents for this purpose [8]: PHMB is the basis for numerous antiseptics. This agent is characterized by good tissue tolerance and a wide range of antibacterial activity [9]. For that reason it has become one of the most commonly used antiseptic agents. The microbicide efficacy of polyhexanide biguanide is based on its effect against acid lipids in bacterial cell membranes, while neutral, human cell membranes are barely attacked [10,11]. The selective activity against bacterial cell membranes defines its good tolerance and wide therapeutic index [10]. Inhibition of bacterial metabolic processes leads to coagulation of the cell content and ends in their death [12,13].

However, the interaction of antiseptics with the wound environment in the clinical setting has not been widely investigated [14,15]. The known extensive concentration of protein in wound exudates suggests it is fundamental importance in wound healing and wound treatment [14–17]. Albumin represents the major fraction of total protein in wound exudates. Thus, the interaction of protein with wound antiseptics should be determined in order to provide proper therapeutic regimens.

The aim of this study was to analyze the antibacterial efficacy of polyhexanide biguanide based agents against *S. aureus* and MRSA in the presence and absence of albumin.

2. Material and methods

2.1. *Quantitative suspension assay*

To evaluate the antibacterial efficacy a standardized quantitative suspension assay (DIN EN 1040) [18] against *S. aureus* (ATCC 6538) and methicillin resistant *S. aureus* (MRSA; ATCC 33592, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig Germany, Artikelnummer: 11729) was performed. Bacterial inhibition testing was performed according to clinical and laboratory Standards, National Committee for Clinical Laboratory Standards, and Deutsches Institut für Normung (58940 Part 3) as described elsewhere.

10^8 colony-forming units *S. aureus* were incubated with different concentrations (0.005–0.1%) of either Prontosan (BBraun Melsungen AG, Melsungen, Germany) or Cosmocil (Arch Chemicals, Ratingen, Germany) in the presence or absence of albumin (albumin solution from bovine serum 30%, Nr.: A 1662, Sigma–Aldrich Chemie GmbH, Taufkirchen, Germany) in ascending concentrations (0%, 0.3%, 0.75%, 1.5%, 3.0%) for 2 min.

In a second approach the effect of increasing concentrations of PHMB (0.04%, 0.1%, 3.0%) in the presence or absence of 3% albumin was investigated for Cosmocil. In addition, the activity against MRSA with increasing concentrations of PHMB and albumin was evaluated. All experiments were repeated on at least six occasions at different days and always compared to untreated controls. Analysis was performed in a blinded fashion.

The effectiveness of the tested products was measured in $\log_{10}$ reduction factor. A decrease of one-unit on the $\log_{10}$ scale indicates a decrease of the antiseptical activity of 10 times, analog two-units on the $\log_{10}$ scale indicate a decrease of 100 times.

2.2. *Statistical analysis*

Data were analyzed by ANOVA and independent *t*-test. The results were presented as mean ± standard error and from $p < 0.05$ on defined as statistical significant, from $p < 0.01$ on highly significant. The Levene test was used for determination of equal or unequal variance. The *t* test for equal or unequal variance was used to detect significance.

Statistic analysis was completed by SPSS 1.0 software (SPSS, Chicago, USA).

3. Results

3.1. *Efficacy of polyhexanid-biguanide-based antiseptics against S. aureus in the presence of albumin*

In the absence of albumin the commercial product Prontosan (0.005% PHMB) reached an antibacterial activity with a reduction factor of 3.8 ± 0.4 $\log_{10}$. A reduction factor of 3.5 ± 0.5 $\log_{10}$ was observed after addition of 0.3% albumin and 3.2 ± 0.4 $\log_{10}$ ($p = 0.019$ vs. 0% albumin) after the addition of 0.75% albumin. A highly significant decrease of the antibacterial effectiveness of only 1.7 ± 0.2 $\log_{10}$ ($p < 0.01$ vs. 0% albumin) was noted for 1.5% albumin. Thus for 3% albumin a reduction factor of 0.8 ± 0.2 $\log_{10}$ was reached ($p < 0.01$ vs. 0% albumin) (Fig. 1).

For Cosmocil (0.005% PHMB) a similar effect could be shown. In the absence of albumin the reduction factor was 4.2 ± 0.3 $\log_{10}$. In presence of 0.3% albumin a significant decrease to 3.6 ± 0.4 $\log_{10}$ ($p = 0.024$ vs. 0% albumin) could be detected. From 1.5% albumin on a highly significant reduction was investigated (2.0 ± 0.3 $\log_{10}$; $p < 0.01$ vs. 0% albumin). Thus for 3% albumin the reduction factor decreased to 0.9 ± 0.2 $\log_{10}$ ($p < 0.01$ vs. 0% albumin) (Fig. 1).

For increasing PHMB at constant albumin-concentrations an increase of the reduction factor was shown. In addition a concomitant decrease of the antibacterial activity in cases of higher albumin could be established even for increased PHMB-concentrations (Fig. 2, Table 1).

3.2. *Antibacterial efficacy versus methicillin resistant Staphylococcus aureus (MRSA)*

For Cosmocil (0.005% PHMB) the antibacterial activity to MRSA was investigated. In the absence of albumin a reduction factor

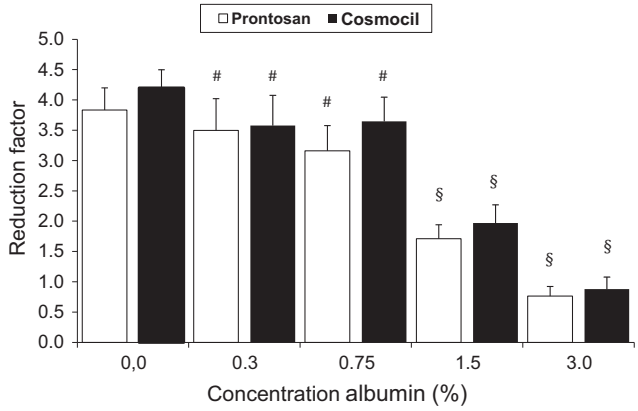

Fig. 1 – Reduction factor depending on the concentration of albumin for Cosmocil and Prontosan with 0.005% PHMB. (# $p < 0.05$ and § $p < 0.01$ compared to a concentration of 0% albumin).

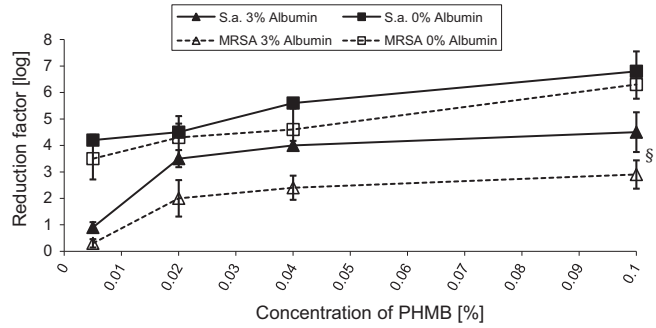

Fig. 2 – The reduction factor for different concentrations of PHMB is given for Cosmocil against *S. aureus* and MRSA. Highly significant differences of the reduction factor were found for concentrations of 3% albumin for MRSA compared to *S. aureus* (§ $p < 0.01$).

of 3.5 ± 0.8 $\log_{10}$ was found. With increasing albumin concentrations a decrease of the reduction factor occurred. For 0.3 and 3% albumin, respectively an effectiveness of 2.8 ± 1.0 $\log_{10}$ and 0.3 ± 0.2 $\log_{10}$ ($p = 0.026$ and $p < 0.01$ vs. 0% albumin) could be detected (Fig. 3). Comparable to the activity against *S. aureus* increasing PHMB concentrations induced an increase of the reduction factor with a concomitant decrease in cases of higher albumin concentrations (Fig. 2, Table 1).

3.3. *Anitbacterial efficacy against S. aureus versus MRSA*

Compared to the antibacterial activity against *S. aureus* the effect of Cosmocil versus MRSA was reduced significantly: in presence of 0 and 0.3% albumin there was no significant difference between the antibacterial activity (0.005% PHMB) ($p = 0.086$ and $p = 0.143$ *S. aureus* versus MRSA). However, for a concentration of 3% albumin we could show a highly significant difference ($p < 0.01$) between *S. aureus* and MRSA (Fig. 2, Table 1). Similarly, this highly significant difference ($p < 0.01$) was found in increasing concentrations of PHMB (0.02%, 0.04%, 0.1%) in the presence of 3% albumin (Table 1).

4. Discussion

To evaluate the antibacterial activity of polyhexanide-biguanide- based antiseptic agents the characteristics of wound exudates play a decisive role. Schmidtchen et al. showed that the antiseptic effect is ensured in presence of wound exudates [19]. Nevertheless in addition to that Rosin et al. documented a reduced activity in those cases [20].

The quantity of albumin varies markedly in acute and chronic wounds. A total protein of up to 5.3% and an albumin concentration of up to 2.7% in the exudates of acute wounds is described [14]. For acute wounds resulting from second degree burns a protein content of 9.3% in the wound exudates could be shown [15]. Chronic wounds contain less protein (total protein 3.7%, albumin 2.1%) [14]. Corresponding results could be found in the ichor of pressure ulcers with 6.5% of protein in total and 2.0% of albumin [16]. The presented in vitro study figures out the effect of albumin to antiseptics and is not able to reproduce all the complex interactions of the wound environment particularly the role of proteases such as neutrophil elastase [17]. However, albumin plays a major role in the wound environment because of the high concentrations measured in wound exudates. Thus a highly-significant loss of antimicrobial effect could be shown in presence of 3%

Table 1 – The antibacterial effect of Cosmocil against *S. aureus* (S.a.) and MRSA for different concentrations of albumin and PHMB.

S. aureus, MRSA: Cosmocil

PHMB (%)	0.005						0.02						0.04						0.1					
Organism	S.a.			MRSA			S.a.			MRSA			S.a.			MRSA			S.a.			MRSA		
Albumin (%)	0	0.3	3	0	0.3	3	0	0.3	3	0	0.3	3	0	0.3	3	0	0.3	3	0	0.3	3	0	0.3	3
Mean	**4.2**	**3.6**	**0.9**	**3.5**	**2.8**	**0.3****	**4.5**	**4.4**	**3.5**	**4.3**	**3.5**	**2.0****	**5.6**	**4.8**	**4.0**	**4.6***	**4.0**	**2.4****	**6.8**	**6.6**	**4.5**	**6.3**	**4.3****	**2.9****
Standard error	0.3	0.5	0.2	0.8	1.0	0.2	0.8	1.0	0.3	0.8	0.7	0.7	0.8	1.1	0.2	0.8	0.8	0.5	0.9	1.0	0.8	0.5	1.0	0.5

*$p < 0.05$ and **$p < 0.01$ differences between the activity against *S. aureus* and MRSA with the same concentration of albumin in the presents of the respective PHMB concentration.
$p > 0.05$ for all results without * or **, which means no significance.
The bold values accent the mean of antiseptical activity.

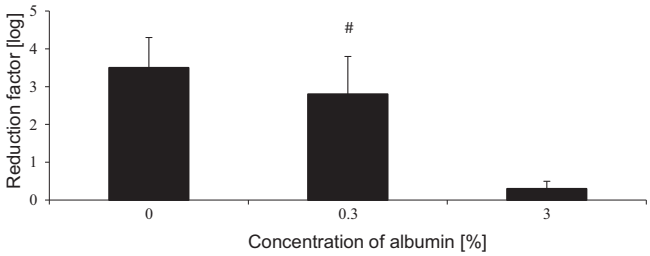

Fig. 3 – Antibacterial activity for Cosmocil (0.005% PHMB) against MRSA (# $p < 0.05$ and § $p < 0.01$).

albumin. Particularly in the treatment of acute wounds, such as burns, an albumin-induced reduction of antiseptic potency of PHMB must be expected.

The results of our study showed that the presence of protein, particularly albumin, reduces the antibacterial activity of polyhexanide-biguanide (PHMB) based antiseptics in a highly significant manner. The loss of antimicrobial effect (decadic reduction factor) is presented as a linear correlation of the rising concentration of albumin. For an application time of 2 minutes and a concentration of 0.005% a significant loss of in vitro activity of Cosmocil could be found starting from concentrations of 0.3% albumin to 3% and for Prontosan from 0.75 to 3% albumin, respectively. When using 1.5% albumin both compounds showed a highly significant loss of efficacy against *S. aureus*.

Comparable results were shown for MRSA. However, antibiotic-resistant *S. aureus* strains possess highly significant, elevated resistance toward PHMB-based local antiseptics in the presence of albumin compared with antibiotic-sensitive control strains. Based on this surprising finding the exact mechanism of the microbiological resistance process needs to be further investigated.

No significant difference in application between both products, the pure polyhexamethylene-biguanide-hydrochloride (PHMB) Cosmocil and Prontosan, including surfactant, could be shown. Nevertheless all investigations presented a mean of the reduction factor for Prontosan lower than for the pure product. As a consequence of this in vitro finding in vivo experiments should pursue whether the combination with a surfactant leads to a clinical reduction of the antiseptic activity of PHMB. Furthermore the effect of albumin on other antiseptic substances should be investigated.

Polyhexanide-biguanide is often used to impregnate wound dressings because of its quality to link to them [21,22]. Due to the occasionally significant fixation to the structure of the dressing only a reduced part of the PHMB is provided as an antibacterial agent. Hirsch et al. showed a dramatically reduction of the effect of clinically used antiseptics and wound irrigating agents in combination with commonly used wound dressings in vitro [23]. Furthermore only 32% of the assessed combinations demonstrated a sufficient antimicrobial activity [23].

Most of the published investigations showing the activity of antiseptics in presence of protein are based on in vitro experiments. However, a wound creates an extremely complex molecular environment. Consequential albumin is only one factor of this molecular environment. More detailed investigations of the numerously elements in acute and chronic wound exudates should clarify their interactions and their effect to antiseptic substances. In addition to wound exudates biofilms play a key role in the pathogenesis of acute and chronical wound infections. This presents a limitation of this study since biofilm-formation was not investigated in this study [17,24]. Thus, we cannot estimate the potential impact of biofilm formation on the antibacterial activity of the antiseptic agents analyzed in this study in the presence and absence of albumin.

With respect to this the limited validity of in vitro studies becomes evident. Nevertheless if in vitro experiments show a significant decrease of antibacterial efficacy in the presence of albumin a sufficient activity in clinical practice may not be expected [22]. Consequential we advise to clean burns properly from wound exudates before application of PHMB-based antiseptics to reduce the shown interference.

Conflicts of interest statement

All named authors hereby declare that they have no conflicts of interest to disclose.

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