

LETTERS TO THE EDITOR

In vitro* Activity of Chlorhexidine and Pentane-1,5-diol and their Combination on *Candida albicans*, *Staphylococcus aureus* and *Propionibacterium acnesNahid Karami^{1,2}, Mikael Alsterholm¹ and Jan Faergemann^{1*}¹Department of Dermatology, and ²Clinical Bacteriology, Sahlgrenska University Hospital, SE-41345 Göteborg, Sweden. *E-mail: jan.faergemann@derm.gu.se
Accepted May 13, 2009.

Sir,

The emergence of multi-resistant bacteria is of great concern in hospital environments and society and it is therefore becoming increasingly important to develop new treatment strategies. Biocides are an alternative to antibiotics because they act non-specifically and against broader targets (1) and bacteria are therefore less likely to develop resistance against biocides (2).

Chlorhexidine digluconate (CHG) is one of the most widely used biocides for antiseptic purposes. It is used in hand-washing (3), oral products and as a preservative (1). CHG is a bactericidal agent with broad-spectrum activity and low skin irritation. Uptake by both bacteria and yeasts is rapid, but its activity is reduced at lower pH and concentration (1). CHG disrupts the outer cell membrane and attacks the bacterial cytoplasm (1).

Diols or glycols are used in pharmaceutical dermatological formulations as solvents, absorptions enhancers and antimicrobial agents (4–6). Pentane-1,5-diol (PD) is a colourless, viscous, odourless agent that is miscible with water (6). It has low oral toxicity and poses little risk of skin and eye irritation compared with other diols (6). PD has high activity against both multi-resistant and sensitive bacteria (7) and against herpes virus and fungi (5). It is a good preservative in various topical formulations and an effective vehicle that can increase the skin absorption of other drugs (4). PD has been shown to have greater antibacterial activity than propane-1,2-diol, a diol that is used widely in dermatology (6).

Propionibacterium acnes is a member of the normal flora of the hair follicle and is also involved in the development of acne vulgaris (8). *Staphylococcus aureus* is the most important bacteria found in various skin infections, including impetigo and secondarily infected atopic dermatitis (8). *Candida albicans* is a member of the normal flora of mucous membranes.

Under the influence of predisposing factors, infections can develop both on mucous membranes and the skin. Invasive life-threatening infections may also develop in immunosuppressed patients (9).

The aim of the present study was to determine the efficacy of CHG and PD and their synergistic effect on the most frequently isolated micro-organisms from the skin by agar dilution method.

MATERIALS AND METHODS

CHG solution, 20% water, C9394-25ML, (Sigma, St Louis, USA) and PD, (BASF AG, Ludwigshafen, Germany) were used in the study and the following reference strains were tested; *C. albicans* (CCUG 5594), *S. aureus* (CCUG 17621) and *P. acnes* (CCUG 1794).

Minimum inhibitory concentrations (MICs) of CHG and PD were determined by agar dilution assay. A series of dilutions of each compound were prepared in blood agar medium for *P. acnes*, and in Diagnostic Sensitivity Test (DST) agar (Oxoid, Basingstoke, UK) for *C. albicans* and *S. aureus*. Dilution series with each agent were made with freshly prepared agar at 48–50°C. Final concentration ranges were as follows: CHG solution, 0.00001 to 1% (w/v) and PD, 1–30% (v/v). The prepared plates were solidified and used immediately.

For each organism an inoculum was prepared by growing organisms on blood agar. Colonies were suspended in phosphate-buffered saline (PBS) and suspensions were adjusted to approximately 6×10^6 colony-forming units (CFU)/ml for *C. albicans* and *P. acnes* and 6×10^5 CFU/ml for *S. aureus*. The plates were inoculated with 20 µl spots and incubated for one day for *C. albicans* and *S. aureus* and 3 days for *P. acnes*. MICs were then determined as the lowest concentration of the compound inhibiting growth of the isolates.

First the MICs of CHG and PD, respectively, were determined for each micro-organism. Then, a range of combined CHG and PD concentrations were tested on each micro-organism to determine if there was synergistic activity. The definition of synergistic effect is: "An effect that is greater than the sum of the effects of the two drugs, such as the equation: $1+1 \dots$ ". Each isolate was tested twice.

Table I. Minimum inhibitory concentration (MIC) of chlorhexidine digluconate (CHG) (w/v %) and pentane-1,5-diol (PD) (v/v %) alone and in combination against *C. albicans*, *S. aureus* and *P. acnes* by agar dilution method. Ratio indicates reduction of the MIC of CHG and PD alone and in combination

Strains	MIC alone		MIC combined		Ratio (alone/combined)	
	CHG	PD	CHG	PD	CHG/CHG+PD	PD/PD+CHG
<i>C. albicans</i> CCUG5594	0.0005	5	0.00005	3	10	2
<i>S. aureus</i> CCUG17621	0.0001	9	0.00001	8	10	1
<i>P. acnes</i> CCUG1794	0.0001	6	0.00005	3	2	2

RESULTS

CHG was effective against all micro-organisms (Table I). MICs of CHG were 0.0005 (% w/v) against *C. albicans* and 0.0001 (% w/v) against *S. aureus* and *P. acnes*.

PD demonstrated an activity against *C. albicans*, *S. aureus* and *P. acnes* of 5, 9 and 6% v/v, respectively. MIC of CHG was much lower than PD with a factor of 1×10^{-4} for *C. albicans*, 6×10^{-5} for *S. aureus* and 9×10^{-5} for *P. acnes* (Table I).

When CHG and PD were combined, in concentrations lower than their respective MICs, their antimicrobial activity increased. MICs of CHG was reduced from 0.0005 to 0.00005 (% w/v) and MICs of PD from 5 to 3 (% v/v) for totally preventing growth of *C. albicans*. MICs of CHG was reduced from 0.0001 to 0.00001 (% w/v) and MICs of PD from 9 to 8 (% v/v) for *S. aureus*. MICs of CHG was reduced from 0.0001 to 0.00005 (% w/v) and MICs of PD from 6 to 3 (% v/v) for totally preventing growth of *P. acnes* (Table I).

DISCUSSION

These results demonstrate that both CHG and PD exhibit antimicrobial activity against the common skin micro-organisms *C. albicans*, *S. aureus* and *P. acnes* when tested by agar dilution assay. The MIC of CHG was much lower than PD, which documents the high efficacy of this agent (10–12). Diols are effective solvents that could target *P. acnes* in the hair follicles and might therefore be effective in the treatment of acne, either alone or in combination with other acne agents.

There have been no reports of the antimicrobial effects of CHG and PD combined, although many studies have demonstrated the synergistic effects and antimicrobial property of CHG when combined with other agents (12–14). Many alcohol products contain low concentrations of chlorhexidine, which remains on the skin. The exact mechanism of action of PD is not fully elucidated, but probably, like other glycols, it acts by dehydrating cells leading to cell death (6–7). These possible interactive effects of CHG and PD may be acted as: (i) targeting the cell membrane by CHG; (ii) drawing water from cytoplasm by PD; (iii) integration with cytoplasmic constituents by CHG.

A study by Karpanen et al. (15) has shown poor permeation of chlorhexidine into the deeper layer (below 300 µm) of the skin, which may restrict the efficacy of skin antisepsis with this agent. PD has been shown to be a good enhancer. The combination of CHG and PD might increase the absorption of CHG into the deeper layer of the skin and can thereby act as an effective skin antiseptic.

In this study *P. acnes* are less inhibited than *C. albicans* or *S. aureus* by combined CHG and PD, although CHG acts at the same MIC level (0.0001%) on *S. aureus* as on *P. acnes*. The reason for this is not evident. It may

depend on differences in the cell membrane structure of the respective micro-organisms.

ACKNOWLEDGEMENTS

This study was supported by ALF-Göteborg.

Jan Faergemann has a patent pending on the use of a combination of pentane-1,5-diol and chlorhexidine in the treatment of bacterial infections. He also has a commercial interest in the development of products with this combination.

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