Protective effect of polihexanide on HaCaT keratinocytes in co-culture with *Staphylococcus aureus*

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Introduction

Staphylococcus aureus is one of the most important pathogen of nosocomial infections and is a common complication during the treatment of chronic wounds. It can exhibit a range of antibiotic resistance (MRSA, *methicillin-resistant Staphylococcus aureus*). Polihexanide (PHMB) is regarded first choice for the antimicrobial treatment of critical colonized or infected chronic wounds because of its good skin tolerability beside its antimicrobial effects. It possesses a specific mechanism of action against acidic lipids of the bacterial membrane and has only little effect on the neutral lipids of the human cell membrane [1]. In fact, Kramer et al. showed that polihexanide promotes wound healing in an animal model [2]. Furthermore, we have investigated the effect of polihexanide on human keratinocytes and found that polihexanide in low concentrations stimulates cell proliferation [3]. Hence, we have used a co-culture system of HaCaT keratinocytes and *Staphylococcus aureus* to test the capacity of polihexanide to protect the cells from the bacterial damage.

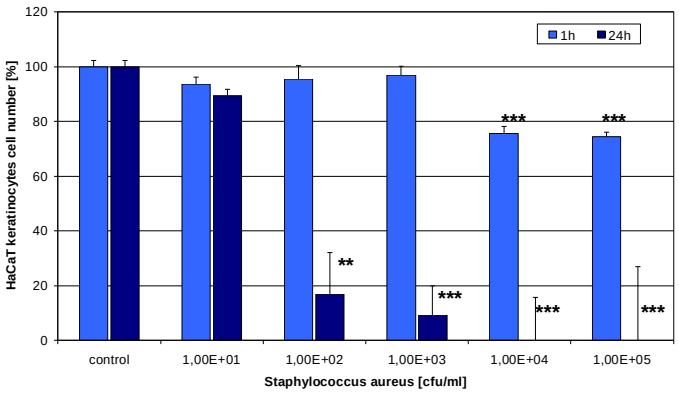

Fig. 1: Negative effect of increasing Staphylococcus aureus concentrations on HaCaT keratinocyte viability and proliferation (measurement of keratinocyte ATP content, mean ± SE).

Material & Methods

HaCaT keratinocytes were cultured with increasing concentrations of *Staphylococcus aureus* and with or without the addition of polihexanide as well as the extract of a polihexanide-containing HydroBalanced biocellulose based wound dressing (PHWD)* in different concentrations. Viability and proliferation of HaCaT keratinocytes was investigated by means of the ATPLite™-M kit (Perkin Elmer). Viable *Staphylococcus aureus* cells were quantified via staining with SYTO-9 (Molecular Probes).

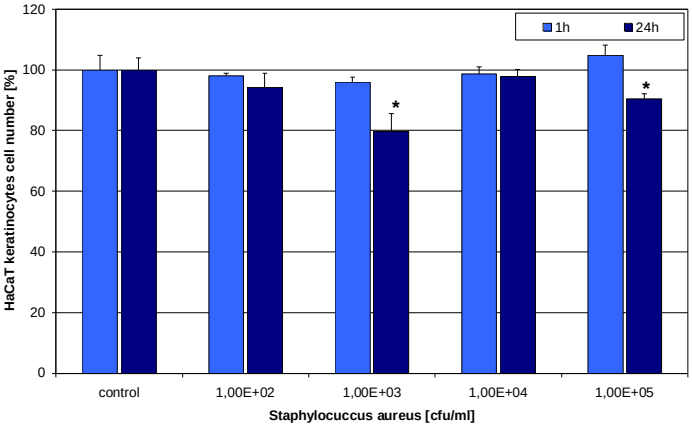

Fig. 2: Addition of 1 µg/ml polihexanide to a co-culture of Staphylococcus aureus and HaCaT keratinocytes protects the cells against bacterial damage (quantification of keratinocytes by measurement of cellular ATP, mean ± SE).

* PHWD = Suprasorb® X+PHMB, Lohmann & Rauscher

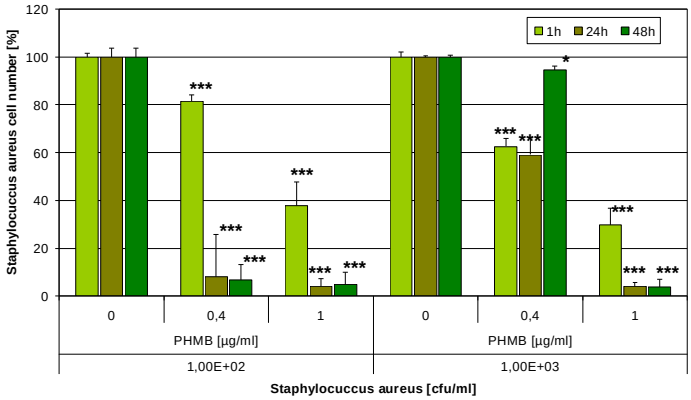

Fig. 3: Polihexanide reduces the number of living Staphylococcus aureus cells and inhibits bacterial growth (staining with SYTO-9, mean ± SE)

Results

Staphylococcus aureus had a concentration-dependent negative effect on HaCaT cell viability and proliferation (Fig. 1). The addition of polihexanide (1 µg/ml) prevented damage to the HaCaT cells and restored normal cell proliferation (Fig. 2). In accordance, the addition of 0.4 µg/ml and 1 µg/ml of polihexanide, respectively, reduced the number of viable bacterial cells as determined via SYTO-9 staining (Fig. 3). Because polihexanide is often used in wound dressings we have investigated the effect of an extract of PHWD* in our co-culture system and observed a significant reduction of *Staphylococcus aureus* growth (data not shown). Hence, the extract of PHWD* was able to protect the keratinocytes from bacterial damage and, furthermore, had a positive influence on the cell proliferation (Fig. 4).

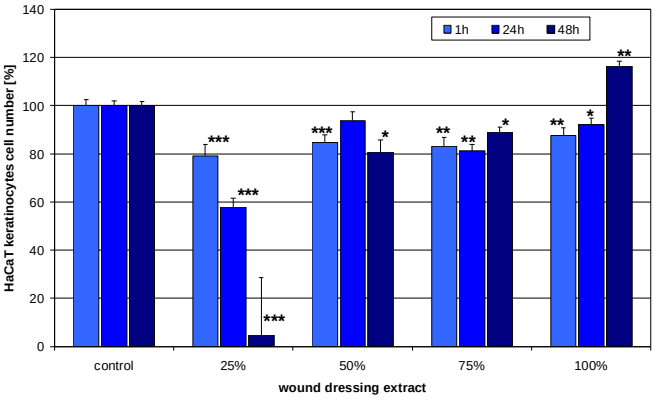

Fig. 4: PHWD extract protects HaCaT keratinocytes in co-culture with Staphylococcus aureus against bacterial damage (determination of HaCaT cell viability via measurement of ATP content, mean ± SE).*

Conclusions

Polihexanide is a highly potent antimicrobial agent, which possesses low cytotoxicity and very good skin tolerability. In addition, it is able to induce cell proliferation *in vitro* [3] as well as in an animal model [2]. Therefore, polihexanide seems to be an ideal antimicrobial substance in wound dressings for treating chronic wounds. Furthermore, we have been able to proof the antimicrobial activity of polihexanide and PHWD* extract in a co-culture of HaCaT keratinocytes and *Staphylococcus aureus in vitro*. It protects the cells from the bacterial damage and allows normal cell growth.

References

1. *Ikeda T. et al. Biochem Biophys Acta 1983; 735: 380-6*
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3. *Wiegand C. et al. GMS Krankenhaushyg Interdiszip 2007; 2(2): Doc43*

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