

Viability and proliferation of fibroblasts, keratinocytes and HaCaT-cells influenced by polihexanide

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Introduction

Wound dressings combined with antimicrobial agents are increasingly utilized in the treatment of critical colonized or infected chronic wounds. Povidone-iodine and octenidine are considered more or less equivalent for disinfecting acute wounds, whereas polihexanide is regarded first choice for chronic wounds because of its good skin tolerance beside its antimicrobial effects. Furthermore, a positive influence of polihexanide on wound closure was observed in individual clinical cases. Therefore we investigated the influence of polihexanide on viability and proliferation of three skin connected cell lines (NHDF, NHEK and HaCaT-cells).

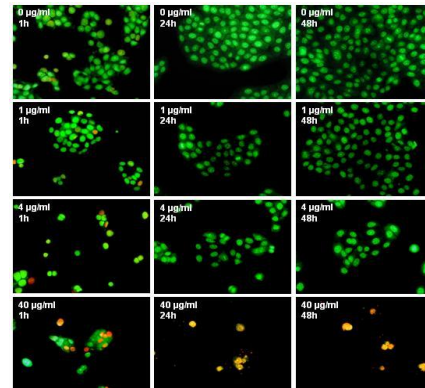


Fig. 3: HaCaT cells stained with SYTO-13 and Ethidiumhomodimer-2 after incubation with polihexanide for up to 48 h. Living cells are dyed green and dead cells red. 400 fold magnification.

Results

The influence of polihexanide on cell proliferation, investigated via measurement of ATP content, is shown in fig. 1. Low concentrations of polihexanide (0.2 – 2 µg/ml) have a proliferative effect on keratinocytes, up to 20% more living cells were found compared to the control. Higher polihexanide concentrations (> 2 µg/ml) displayed a negative effect on proliferation of all cell types. These results were supported by microscopic evaluation of the area covered by cells after staining with SYTO-13 and EthD-2 (fig. 2). Moreover, the live and dead staining revealed a significant increase of dead cells for 40 µg/ml polihexanide. Surprisingly the medium concentrations of 4 µg/ml showed almost 100 % living cells but a significant lower cell number compared to the control was observed by ATP assay and microscopic evaluation (fig. 3 and fig. 4).

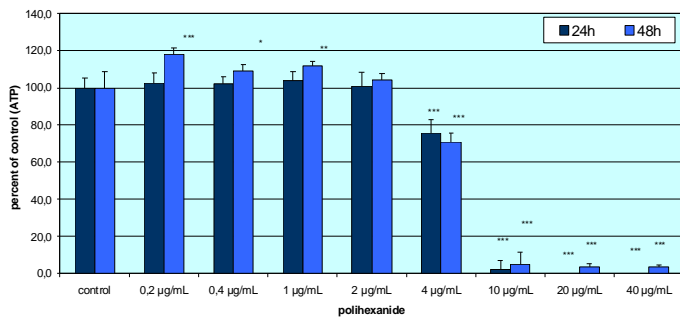


Fig. 1: Influence of polihexanide on keratinocyte proliferation (NHEK) determined via measurement of the ATP content (mean ± SE).

Material and methods

Viability and proliferation of normal human dermal fibroblasts (NHDF), normal human epidermal keratinocytes (NHEK) and HaCaT-cells (human adult high calcium low temperature keratinocytes) were investigated via microscopic evaluation of live and dead cells (analySIS® 3.1 Soft Imaging System GmbH, Germany) and by means of the ATPLite™-M kit (Packard Bioscience BV, The Netherlands). To distinguish between live and dead the cells were stained with SYTO-13 and Ethidiumhomodimer-2 (Molecular Probes, U.S.). The luminometric ATP assay is based on the detection of light generated by the ATP dependent enzymatic conversion of D-luciferin by luciferase.

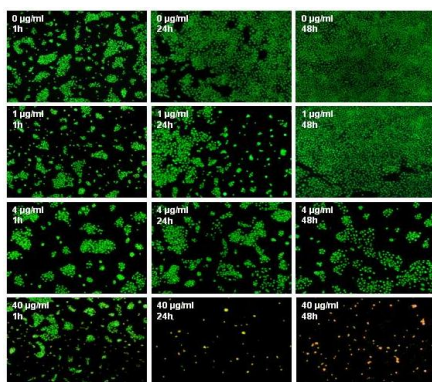


Fig. 2: HaCaT cells stained with SYTO-13 and Ethidiumhomodimer-2 after incubation with polihexanide for up to 48 h. Living cells are dyed green and dead cells red. 100 fold magnification.

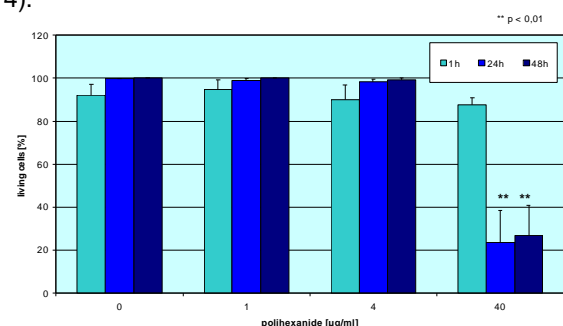


Fig. 4: Influence of polihexanide on HaCaT cell viability. Living cells (green) in percent compared to total amount of cells (green + red) by microscopic evaluation of pictures as shown in fig. 2 (mean ± SE).

Conclusions

A significant increase of the proliferation of human keratinocytes and HaCaT-cells by polihexanide was found. In higher concentrations a dose-dependent decrease of foremost proliferation and then cell viability was observed. These *in vitro* observations are in accordance with *in vivo* studies recently published (Kramer et al. 2004) and demonstrate an impressive positive influence of polihexanide on the proliferation of different cell types. Therefore polihexanide seems to be an ideal antimicrobial substance in wound dressings for treating chronic wounds because of its low cytotoxicity, good skin tolerance and positive influence on proliferation.