An In Vitro Wound Healing Model Assessing Wound Healing Properties of Benzenthionium Chloride Bioclay

Jerome Kiripolsky, Dr. Paul Kostyniak, Dr. Patricia Masso-Welch, Department of Biotechnical and Clinical Laboratory Sciences, School of Medicine and Biomedical Sciences, University at Buffalo, 3435 Main Street, Buffalo, NY 14214

Abstract

Benzenthionium chloride (BTC) is a quaternary ammonium compound (QAC) that is used as an antibacterial in several applications. BTC can be complexed with laorone clay (Bioclay) particles, making it capable of being applied and retained typically. Once complexed, BTC cannot be easily leached out of the clay by diffusion due to strong interactions with the clay surface. Bioclay has a wide range of potential applications for imparting antimicrobial activity in medical, industrial, and household applications. Bioclay has been previously shown to be effective against methicillin resistant Staphylococcus aureus (MRSA), and has been developed in cream form for topical application as an antifungal cream. This study investigates the hypothesis that BTC, when incorporated into a pharmacological grade scaffold, can enhance the process of wound healing. This is done using an in vitro wound healing model utilizing a variety of cell lines. WI38 cells are normal diploid human fibroblasts. TM12 and TM12T are immortalized mammary epithelial cell lines; TM12T is a derivative of TM12 and has undergone epithelial to mesenchymal transformation. The first study utilized the TM12 cells which were harvested in suspension and centrifuged. The cells were washed with PBS before the different medias were added. In this study an induced ‘wound’ was formed using media treated with either BTC or Bioclay. The wound width was measured after 24 hours to compare the width of the wounds between the control and treatment groups. A viability assay was run in parallel, to control for any effects of BTC compounds on total viable cell number. No effect was seen on wound healing at any of the concentrations tested, in either fibroblasts, epithelial or epithelial cell lines. Lack of effect was not due to loss of viability. Although no effect was seen of BTC compounds on these purified cell lines, these findings may be affected, due to the co-opted participation of several cell types, including granulocytes, macrophages, fibroblasts, and epithelial cells.

Introduction

Wound beds are complex environments in which dead tissue, exudate, and the bacterial biofilm interact in a complicated manner among themselves as well as the host. In these situations, bacteria tend to organize into biofilms, reducing their susceptibility to elimination as well as impairing healing. The cleaning of the wound is one of the basics of wound healing and can be detrimental to the wound bed. Published literature shows that cleaning improves the wound environment and accelerates healing. In previous studies, BTC has proven to be effective against a number of bacteria including (but not limited to) E. coli, H. influenzae, S. typhi, S. aureus, and S. pneumoniae. Aremidal evidence from topical use of Bioclay has indicated that BTC complexed with laorone can have a positive effect on wound healing. This is a first attempt to understand the mechanism(s) by which this can occur.

Methods

Wound healing assays: Cells were plated in 24 well plates at 5x10^4 cells/well and grown to confluence. The confluent layer of cells in triplicate wells was then scratched from the top of the well down using a micropipette tip. Wells were rinsed with sterile PBS to remove detached cells. Cells were then fed with media containing 0, 1.25, 2.5, or 5 µg/ml BTC or Bioclay. These concentrations were selected to be below concentrations previously used in a cream formulation (50 µg/ml), and based on literature that assessed the toxicity of BTC. At 24 hours, images were taken using a phase contrast inverted microscope. Wound diameter was measured at the start point (D0) and at 24 hours to assess % wound closure at 24 hours using the formula:

\[ \text{Wound Closure} \times 100 \]

Results

Wound beds are complex environments in which dead tissue, exudate, and the bacterial biofilm interact in a complicated manner among themselves as well as the host. In these situations, bacteria tend to organize into biofilms, reducing their susceptibility to elimination as well as impairing healing. The cleaning of the wound is one of the basics of wound healing and can be detrimental to the wound bed. Published literature shows that cleaning improves the wound environment and accelerates healing. In previous studies, BTC has proven to be effective against a number of bacteria including (but not limited to) E. coli, H. influenzae, S. typhi, S. aureus, and S. pneumoniae. Aremidal evidence from topical use of Bioclay has indicated that BTC complexed with laorone can have a positive effect on wound healing. This is a first attempt to understand the mechanism(s) by which this can occur.

Discussion

• There was no enhancement of wound healing responses in either WI38, TM12 or TM12T cells by BTC or Bioclay.
• There was no difference between the BTC and Bioclay groups for any of the cell lines used. The TM12 cell line was more sensitive to the BTC and Bioclay than the TM12T cell line.
• WI38 fibroblast viability was more sensitive to Bioclay than BTC alone as seen in the viability assay.
• At higher concentrations of BTC there was a slight loss of cell vitality in TM12 cells, but TM2T cells were less sensitive to BTC because they were immortalized.
• There is no decrease in cell viability (with a P < 0.05) except for the 5.0 µg/ml Bioclay treatment group. The 5.0 µg/ml Bioclay group was different from all the other groups with a P-value of at most 0.006. All of the other groups are statistically similar.

References

3. Ig et al., 2006 Cancer Res. 66(24):5575-5580