SIGNIFICANT AND RAPID REDUCTION OF FREE ENDOTOXIN CONCENTRATION BY DIALKYLCARBAMOYL CHLORIDE (DACC)-COATED WOUND DRESSING

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Introduction

Endotoxin or Lipopolysaccharide (LPS) is a component of the outer cell membrane of the gram-negative bacteria, and is mainly released when the bacteria grow, die or damaged. The lipid A domain of endotoxin is responsible for its toxicity.

Endotoxin is a known potent trigger for inflammation. Several *in vitro* studies suggest that endotoxin also contributes to a delay in wound healing [1-4]. Therefore, reduction of endotoxin in the wound may lead to less inflammation and better wound healing process.

Dialkylcarbamoyl chloride (DACC)-coated wound dressing reduces bio-burden in the wound by hydrophobic binding of microorganisms. It is hypothesized that the hydrophobic interaction also occur between the DACC-coated wound dressing and the hydrophobic Lipid-A part of endotoxin.

Aims

1) To explore the ability of DACC-coated wound dressing to bind endotoxin from *Pseudomonas aeruginosa in vitro*.

2) To investigate its effect on the level of endotoxin released from gram-negative bacteria.

Materials & Methods

For endotoxin binding experiment, two punched circular (14 mm Ø) DACC-coated wound dressing (Sorbact ® Compress) were incubated with of 50 µl of purified *P. aeruginosa* endotoxin solution at different concentrations for various durations up to 48 h at 37°C, followed by vigorous vortexing for 1 minute. After removal of he wound dressing pieces, the samples were analysed for endotoxin.

To investigate the effect of DACC-coated wound dressings on the level of endotoxin released from gram-negative bacteria, another two punched circular pieces (14mm Ø) of DACC-coated dressing (Sorbact ® Compress) were incubated with 50 µl of 10^8 CFU/ml *P. aeruginosa* for 1 hour at 37°C. After incubation, intact bacteria were separated by centrifugation and filtration. The supernatants were analyzed for endotoxin.

Endotoxin analyses were performed using Limulus assay.

Results & Discussions

In this *in vitro* study, DACC-coated dressing was able to consistently reduce endotoxin concentration by 93-99% ($P \leq 0.0001$) after 24 h. Even at a very high endotoxin concentration of 11000 EU/ml, 99% reduction can be seen after 24 h incubation (Fig. 1). A significant endotoxin reduction of 39% ($P \leq 0.001$) was observed already at 5 minutes, and continued over time to 48 h (Fig. 2). Moreover, endotoxin that bound to the dressing adhered strongly, given that it was not released by the extensive vortexing for 1 minute.

After a one-hour incubation of clinically relevant *P. aeruginosa* strain with DACC-coated dressings, no increase of free endotoxin concentration was observed. Instead, free-endotoxin concentration was reduced to below detection limit (from 420 EU/ml to <0.2 EU/ml, >99.95% reduction).

Conclusion

This is the first study to show that DACC-coated wound dressing is able to significantly and rapidly bind purified and shed endotoxin from *P. aeruginosa*. This ability to remove both endotoxin and bacterial cells may lead to less inflammation in the wound and better wound healing process.

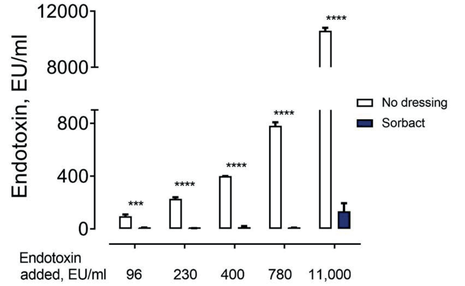

Figure 1: Binding of purified *P. aeruginosa* endotoxin by DACC-coated dressing. Endotoxin remaining in the medium after overnight incubation with the DACC dressing discs was analyzed by a quantitative Limulus assay. Significant differences are indicated by asterisks, ***P ≤ 0.001, ****P ≤ 0.0001.

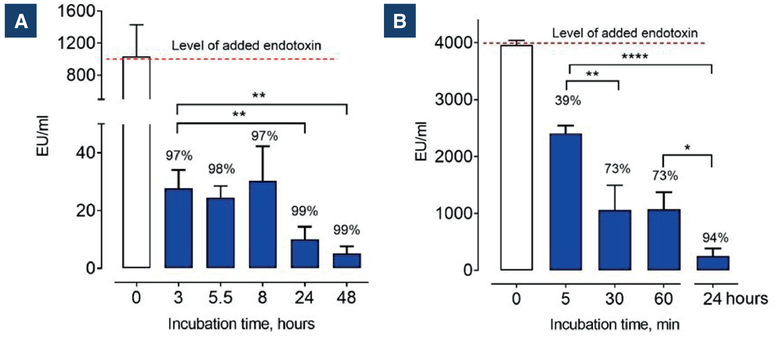

Figure 2: Kinetics of binding of purified *P. aeruginosa* endotoxin to DACC-coated dressing. Endotoxin remaining in the medium was analyzed at various time points during incubation with the dressing discs and performed at 1000 (A) and 4000 (B) EU/ml of endotoxin. The unfilled bars in A and B represented the initial concentration at the start of incubation. Significant differences are indicated by asterisks, *P ≤ 0.05, **P ≤ 0.01, ****P ≤ 0.0001.

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