R031
Publication Only
Basic Science: Biofilm

Utilisation of ionic liquids for pathogen neutralisation

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Objectives:

Biofilms formed by pathogenic bacteria show increased resistant to antibiotics because the antibiotic cannot penetrate the extracellular polymeric substances (EPS). Ionic liquids (ILs) or deep-eutectic solvents (DES) are increasingly recognized as suitable materials for multiple biological applications. Based upon the tunable nature of the ILs, by varying both the anionic and cationic component, the formulations result in differing physicochemical properties of the material. As such, this project aims to develop and optimize therapeutic formulations (ILs) that have anti-biofilm properties. In addition, the identical ILs were assessed for irritation, permeation (transdermal delivery), and cytotoxicity properties against human undifferentiated lung cells and skin.

Methods:

The gram-negative bacteria, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar *typhimurium* LT2, were employed for the biofilm disruption studies. The bacteria were grown in MBEC plates as either 24 or 72 hour biofilms using the Calgary method. Each biofilm-containing well was challenged with an IL previously synthesized and characterized in our laboratory, for two hours, then the IL was removed by washing and viability determined by enumeration. IL-induced cytotoxicity and cellular proliferation on normal human bronchial epithelial (NHBE) cells were assessed using the LDH and WST-1 assays, respectively. Franz Diffusion Cells were used to determine transdermal penetration properties of either tritium-labeled mannitol or cefadroxil formulated into each IL in our panel. The stratum corneum, epidermis, and dermis were isolated post 24 hour IL exposure and the radiolabeled material quantified by scintillation counting.

Results:

For each IL/DES formulation, the physicochemical properties were determined and include partition coefficients, conductivity, viscosity, density and ionic strength. These values were integral for determining whether correlations exist for biofilm disruption and pathogen neutralization, skin toxicity, and skin penetration/drug delivery. All of the IL/DES tested exhibited good to excellent anti-biofilm properties (>99% cell death), however, most of the solvents were rather toxic to the cell lines and/or had poor transdermal penetration properties. We identified one DES, choline-geranic acid (IL-21), that was able to kill >99.99% of the bacteria found within both the 24 and 72 hour biofilms after only a brief 2 hour exposure. Further, this same DES exhibited excellent transdermal penetration with greater than 25% of the antibiotic found in the epidermis, dermis, or acceptor solution (complete passage). Finally, IL-21 had minimal cytotoxicity and cellular proliferation effects on NHBE cells.

Conclusions:

Our studies focused on the gram negative pathogens, *Salmonella enterica* serovar *typhimurium* and *Pseudomonas aeruginosa* when grown as biofilms over either 24 or 72 hours. We tested a panel of IL/DES and found most have excellent anti-biofilm properties but, unfortunately, have poor skin permeation properties and/or are toxic to human cell lines. We identified one DES, choline-geranic acid,

which exhibited all the desired properties: antimicrobial agent, minimal cellular cytotoxicity, and transdermal drug delivery.