Evaluation of Antibiofilm Activities of Some Drug Combinations Against Wound Bacterial Pathogens

Thesis
Presented in fulfillement of requirements of

Ph. D. Pharmaceutical Sciences
(Pharmaceutical Microbiology)

Presented by

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(2003)

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2011

Abstract

It has been hypothesized that biofilms play a role in the prevention of wound healing. Putting into consideration biofilm resistance to antimicrobial agents which is often due to slow impaired antibiotic penetration, altered micro environments and slow growth adaptive response and presence of persisters.

As this problem is greatly encountered in Egypt specially in post surgical facilities , the present study aimed at assessment of biofilm implicated wound infections and treatment options through isolation and identification of infected wound associated pathogens, determination of biofilm formation by the isolated pathogens, studying interspecies interaction that may affect biofilm formation, determination of antimicrobial resistance pattern of selected biofilm producing isolates, determination of activity of different drugs on the selected pathogens, studying the effects of drug combinations on the formation of single and mixed bacterial biofilms as well as their effects on infected induced wound in rats (*in vivo* models).

Swabs were taken from 52 different wounded subjects. A total of 80 isolates were obtained, revealing both mixed and single isolates that were further examined for their biofilm forming capacities. Moreover, determination of some virulence factors production by *Pseudomonas* species that could affect biofilm formation and that with biofilm formation are documented to be controlled by quorum sensing phenomena. Virulence factors were also determined among *Staphylococcus* isolates. Also, determination of presence of N–acyl–homoserine lactone in all tested *Pseudomonas* isolates and correlation of its presence with certain virulence traits and biofilm formation. Furthermore, susceptibility testing of all isolates was done.

Assay of antibiofilm effect in the form of prevention of biofilm formation by a range of antibiotic concentrations including sub inhibitory concentrations was done. In addition, effect of dexpanthenol as wound healing agent on MIC of the topically applied chosen antibiotics and on their biofilm inhibition ability as well as its own ability to inhibit growth and adhesion of bacteria, Detection of Efflux pump inhibition capacity of dexpanthenol was detected using ethidium bromide.

Determination of spectrum of activity of propolis as a natural known healing agent with antimicrobial activity, its MIC against selected pathogens, its effect on activity of some antibiotics as detected by diameter of inhibition zone

and its ability to prevent biofilm formation was also performed. Finally, *In vivo* study was done on six groups of rats using two (antibiotic–dexpanthenol) combinations to determine their effect on wound healing as assessed by histopathological examination.

Study revealed that *Staphylococcus* followed by *Pseudomonas* species were the most commonly isolated organisms from wounds. Biofilm forming capacity was the highest among both *Pseudomonas* and *Staphylococcus* isolates Presence of *Pseudomonas* and *Staphylococcus* in mixed culture had a great effect on the formed biofilm mass by either positive or negative impact. Virulence factors appeared to be implicated in the biofilm formation and were correlated to the production of N–acyl–homoserine lactone by all *Pseudomonas* isolates. Multidrug resistance especially to β lactams, aminoglycosides and quinolones was noticed among wound isolated pathogens. Shared resistances to erythromycin, β lactams and quinolones were observed among *Staphylococcus* and *Pseudomonas* in co–culture.

Dexpanthenol, although showing no antimicrobial action by its own, in concentration used in this study had a great effect on the MIC of most of the tested antibiotics (potentiated their activity), the antibiofilm activity of dexpanthenol alone was high and comparable to that of antibiotics (as subinhibitory concentration affected bacterial adhesion). Furthermore, the dexpanthenol showed a mild efflux inhibition effect. Propolis showed a marked effect on antibiotic activity, such effect differed in case of Gram positive and Gram negative bacteria. Moreover, testing antibiofilm activity of propolis extract against the isolates in mixed and single culture revealed a great decrease in adhesion in its presence at low concentrations.

Induction of wound infection in rats demonstrated, biofilm formation in twenty four hours and resolution of infection and underlying inflammation by antibiotic and antibiotic dexpanthenol combinations treatment compared to dexpanthenol alone and control groups. Scare-less wound healing was noticed in all antibiotic and antibiotic-dexpanthenol treated rats.

Therefore, we can conclude antibacterial action of the topically applied antibiotics is improved greatly in presence of dexpanthenol which could be formulated in the same vehicle and administered locally to assist in prevention of surgical site infections putting into consideration the antibiofilm activity of dexpanthenol. Propolis Extract as an old natural remedy could still be of use as local wound management, being used alone or with the suitable antibiotic to ensure maximum benefit.