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Characterisation of the synergistic vancomycin-furazolidone action against *Escherichia coli*.

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Abstract

The use of antibiotic combinations is garnering increased interest in the recent years due to the spread of antibiotic-resistant bacteria. The shortage of antibacterial therapy options is particularly severe for infections caused by Gram-negative bacteria, due to the formidable barrier to molecules > 600 Da imposed by the outer membrane. Vancomycin is a large glycopeptide antibiotic to which the outer membrane is poorly permeable, hence the minimal inhibitory concentration of this antibiotic for *Escherichia coli* is very high (~500 mg/L). Due to the resistance of *E. coli* and other Gram-negative pathogens to an increasing number of < 600 Da antibiotics including beta lactams, aminoglycosides and quinolones, enabling vancomycin use on Gram-negative bacteria would be valuable. Furazolidone was reported to increase sensitivity of *E. coli* to vancomycin, and this interaction has been investigated in this thesis in order to explore the potential of the vancomycin-furazolidone combination for clinical applications. The initial analysis of the vancomycin-furazolidone synergy demonstrated that their interaction is synergistic rather than merely additive. Furthermore, effectiveness of this combination for growth inhibition and eradication of *E. coli* biofilm was investigated. However, despite the synergy between vancomycin and furazolidone, the concentration of vancomycin in combinations required for growth inhibition and killing of *E. coli* in a planktonic mode and as a biofilm was above the nephrotoxicity (toxicity in the kidneys) threshold and therefore too high to treat infections with this organism systemically. However, by adding deoxycholic acid to the combination, the bactericidal vancomycin concentration was decreased below the nephrotoxicity threshold. The mechanism of synergy in the planktonic mode of growth was investigated through the analysis of *E. coli* gene-knock-out mutants and it was observed that TolC, the outer membrane channel common to a number of efflux systems (exporting enterobactin, xenobiotics and metabolites) is likely to be involved in vancomycin-furazolidone synergy. However, it was not possible to reliably pinpoint any particular efflux pump or enterobactin accumulation as factors in synergy. Using the genetic approach, it was found that DNA excision repair endonuclease UvrABC was ruled out as a factor involved in synergy. Overall this study characterised the synergy between vancomycin and furazolidone, initiated the enquiry into the mechanisms of interaction between these two antibiotics and examined its effectiveness against biofilms.

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Abbreviations

%	Percentage
$	Dollars
°C	Degrees Celsius
µL	Microlitre
µm	Micrometre
8-OHdG	8-hydroxydeoxyguanosine
ABC	Adenosine triphosphate binding cassette
AMR	Antimicrobial resistance
ATP	Adenosine triphosphate
$CaCl_2$	Calcium chloride
CAUTI	Catheter associated infections
CDC	Centres for Disease Control and Prevention
CFU/mL	Colony forming units per millilitre
Da	Daltons
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOC	Sodium deoxycholate
ECM	Extracellular matrix
ESBL	Extended-spectrum beta-lactamase
FICI	Fractional Inhibitory Concentration Index
g	Grams
g/L	Grams per litre
HepG2	Hepatoma

kDa	Kilodaltons
Km^R	Kanamycin resistance marker
kPa	Kilopascals
LPS	Lipopolysaccharide
MATE	Multidrug and toxic efflux
MBC	Minimal Bactericidal Concentration
MBEC	Minimal Biofilm Eradication Concentration
MBIC	Minimal Biofilm Inhibitory Concentration
mg/kg	Milligram per kilogram
mg/kg/day	Milligram per kilogram per day
mg/L	Milligram per litre
$MgSo_4$	Magnesium sulfate
MIC	Minimal Inhibitory Concentration
mL	Millilitre
mM	Millimolar
MRSA	Methicillin resistant *S. aureus*
Na	Sodium
nm	Nanometre
NO	Nitric oxide
NZ	New Zealand
OD	Optical density
ORF	Open reading frame
PMF	Proton motive force
RND	Resistance nodulation division

Rpm	Revolutions per minute
ROS	Reactive oxygen species
SOC	Super Optimal broth with Catabolite repression
SMR	Small multidrug resistance
USA	United States of America

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