Minocycline and rifampicin combination therapy for treatment and prevention of emergence of resistance in MRSA

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Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is a leading cause of serious community- and hospital-acquired infections. Resistant isolates have been recognised rapidly after introduction of new anti-staphylococcal agents; within 2 years of introducing methicillin, linezolid, and daptomycin. Interestingly vancomycin-resistant isolates were only recognised 40 years after its introduction and are still rarely isolated, however in recent years decreased susceptibility has been reported in many first-line therapies including vancomycin

Rifampicin inhibits DNA-dependent RNA synthesis and has been used in combination with glycopeptides to treat MRSA infections successfully, particularly where deep tissue penetration is required such as in endocarditis, osteomyelitis, joint infections. The disadvantage of rifampicin is its predisposition to rapid emergency of resistant isolates meaning it must be used in combination with another antimicrobial. Concurrent rifampicin therapy is currently the subject of a clinical trial in all-cause MRSA bacteraemia.[1]

Given the decreasing susceptibility of MRSA to first-line antimicrobials, it is necessary to investigate alternatives. Long-acting tetracyclines such as minocycline have high oral bioavailability, tissue penetrance, and *in-vitro* anti-staphylococcal activity including against MRSA. Minocycline also exhibits synergy with rifampicin *in-vitro* against MSSA and MRSA.

A murine PK-PD study was performed to examine the combination of minocycline and rifampicin for MRSA infection.

1. Thwaites G, et al. *Trials* 2012.

2. Greco W, et al. *Pharmacol Rev* 1995.

Methods

A murine thigh-infection model of MRSA was used. Mice were infected with 1 x 10^5 CFUs of MRSA strain 43456 in each thigh. Treatment commenced 2 hours post-inoculation with combinations of minocycline 10, 25, and 75 mg/kg/day Q6h and Q8h IV and rifampicin 10, 50, and 100 mg/kg/day q24h IV. Mice were sacrificed at 26, 50, 74, and 98 hours post-inoculation.

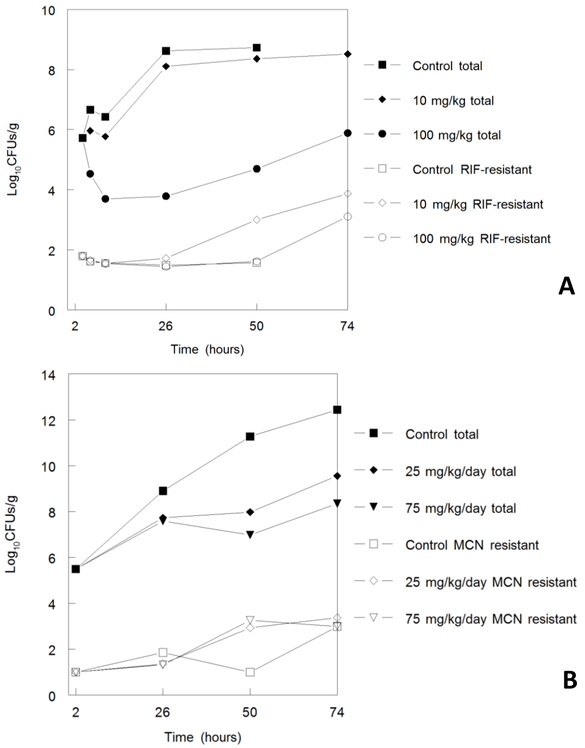
Thigh homogenates were quantitatively cultured on CLED media, CLED containing 32 mg/L minocycline, -containing and CLED containing 8 mg/L rifampicin. A final set of plates containing both 32 mg/L minocycline and 8 mg/L rifampicin was used.

Minocycline and rifampicin plasma concentrations were analysed using LC/MS/MS. Concentrations were determined at 0.5, 2, 4, 6, and 26 hours after a single IV dose in separate experiments.

A computational model using Pmetrics was employed to examine the effect of drug-exposure on susceptible MRSA killing and the emergence of resistance. Drug-drug interactions were modelled using the Greco URSA model[2] implemented within the differential equations. Outputs included each drug concentration in plasma, susceptible, rifampicin-resistant and minocycline-resistant CFUs/g in thigh homogenates.

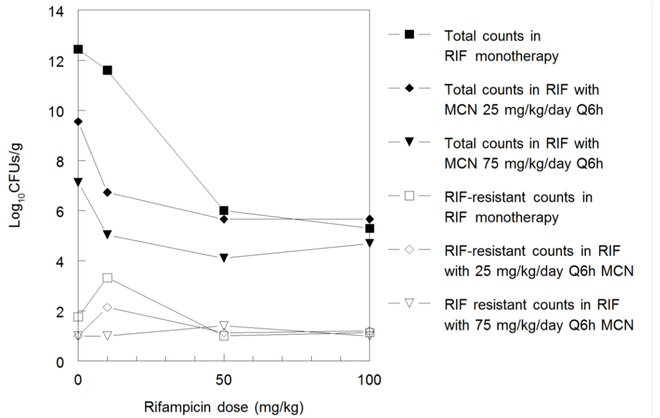
Results

Individual posterior fits of the model to both PK and PD data were satisfactory. Rifampicin and minocycline were synergistic in killing susceptible MRSA (alpha=3.10, CI=2.71, 3.51) and minocycline-resistant MRSA (alpha=3.8, CI=3.43, 4.26) but antagonistic in suppression of rifampicin-resistant MRSA (alpha=-3.00, CI=-2.51, -3.49).



A Time-course of susceptible and resistant CFUs/g MRSA in 10 and 100 mg/kg rifampicin (RIF) Q24h.

B Time-course of susceptible and resistant CFUs/g MRSA in 25 and 75 mg/kg/day minocycline (MCN) Q6h.



CFUs/g MRSA at 74 hours across rifampicin (RIF) doses with monotherapy, 25 mg/kg/day and 75 mg/kg/day Q6h minocycline (MCN)

PK-PD relationships were not examined on resistance to both rifampicin and minocycline since few isolates were observed. All treatment groups exposed to rifampicin as monotherapy or in combination lead to amplification of rifampicin-resistant mutants. This was incompletely suppressed with the addition of minocycline.

Conclusions

Minocycline and rifampicin combination therapy in a murine thigh-infection model of MRSA shows *in-vivo* synergy in killing susceptible isolates but rifampicin resistance emerged in all treatment groups exposed to rifampicin.