Impact of MTN Inhibitors on drug resistance in Methicillin-Resistant Staph. aureus (MRSA)

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) infections account for approximately half of healthcare-associated multidrug-resistant pathogen infections and result in about 19,000 deaths per year in the United States. Thus, development of new antibiotics to treat this pathogen is important. Methylthioadenosine-5-adenosylhomocysteine nucleosidase (MTN) is a biologically significant bacterial enzyme responsible for the catabolism of nucleosides produced through a variety of pathways. The cellular accumulation of these nucleosides limits bacterial growth through negative feedback mechanisms. MTN is also involved in autoinducer signaling in quorum sensing that regulates bacterial growth, biofilm formation, virulence, and drug resistance. Our experimental data shows that although MTN inhibitors do not show strong antibacterial effects by themselves, potent MTN transition state analogs can work to improve MRSA and Staph. sensitivity to standard antibiotics when given in combination. Thus MTN inhibitors could be used to reverse drug resistance.

Background

The bacterial specific enzyme MTA/SAH nucleosidase (MTN) is responsible for the catabolism of nucleosides that are produced by several metabolic pathways. When accumulated, these nucleosides act as product inhibitors and growth regulating molecules. MTN is essential for salvage of purines and methionine, and is connected to autoinducer signaling that regulates bacterial growth, biofilm resistance, and drug resistance. This enzyme serves as a prospective target for the development of new antibiotics.

Hypothesis / Rationale

Staphylococcus aureus and MRSA drug sensitivity profiles will be altered by treatment with MTN inhibitors. Potential Mechanisms:
- Interruption of quorum sensing dependent drug resistance
- Interruption of MTN / methionine salvage related pathways:
  - Folate metabolism (trimethoprin target)
  - Glutathione / Antioxidant related responses (H2O2 sensitivity).

Experimental Approach

- S. aureus ATCC 6538 and MRSA-1 incubated at 37°C overnight in MH broth
- Overnight cultures diluted to 0.5 McFarland standard turbidity, then further diluted 1:100 by volume
- 96-well Plate Setup (4 replicates):
  - 20 µL of drug
  - 80 µL of media
  - 100 µL of diluted culture
- Negative controls consisted of media only and Sodium azide-killed cells
- Absorbance values (600nm) measured with a microplate spectrophotometer
- Drug sensitivity measured alone and in combination with other antibiotics

Conclusions

- MTN inhibitors are poor anti-Staph agents by themselves.
- MTN inhibitors have the potential to be used in conjunction with antibiotics to increase the sensitivity of MRSA to standard drugs.

Future Studies

- Biofilm formation studies
- Clone, express, and purify Staph. aureus MTN enzyme
- Mechanistic studies on interaction of MTN inhibitors and other drugs
- Enzyme kinetics studies

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Fig. 1: Several metabolic pathways produce MTN substrates

Fig. 2: 96-well plate schematic

Fig. 3: Drug sensitivity profiles: A) Oxacillin, B) Trimethoprin, C) H2O2

Fig. 4: Table of drug IC50 for S. aureus and MRSA

Fig. 5: Inhibition of A) MRSA-1 and C) S. aureus by MTD

Fig. 6: Drug Synergy Studies. Transition state analogs improve drug sensitivity to Oxacillin, Trimethoprin and H2O2.