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Evaluating the Antimicrobial Activity of Esomeprazole Magnesium Trihydrate on Antibiotic-Resistant Bacteria Using Efflux Pumps

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Funding: No specific funding was received for this work.

Potential competing interests: No potential competing interests to declare.

Abstract

Background: Antibiotic resistance mediated by efflux pumps is a serious public health threat. Esomeprazole magnesium trihydrate (EMT) is a proton pump inhibitor with reported antimicrobial activity, but its effects against efflux-mediated resistance are unknown.

Objective: To evaluate the antimicrobial activity of EMT alone and in combination with the efflux pump inhibitor reserpine against clinical isolates of *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Staphylococcus aureus*.

Methods: Minimum inhibitory concentration (MIC) assays were performed using the broth microdilution method on 15 non-duplicate clinical isolates and reference strains with/without EMT and reserpine.

Results: EMT demonstrated activity against all isolates, with MIC ranges of 4-32 µg/mL, 8-64 µg/mL and 2-16 µg/mL respectively. EMT MICs decreased 4-8-fold for 13/15 isolates when combined with reserpine, indicating EMT may inhibit efflux pumps. Similar reductions occurred for comparator antibiotics.

Conclusions: This study provides the first evidence that EMT possesses intrinsic antimicrobial activity against these pathogens and may function as both an efflux pump substrate and inhibitor. EMT warrants further investigation as a potential adjuvant antibiotic for overcoming efflux-mediated resistance.

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Keywords: antibiotic resistance, efflux pumps, esomeprazole magnesium trihydrate,*Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*.

Introduction

Antibiotic resistance poses a serious global threat, as many bacterial pathogens have developed resistance to multiple antibiotic classes. Efflux pumps are an important resistance mechanism, as they actively expel antibiotics from bacterial cells. Major pathogens like *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Staphylococcus aureus* commonly express multidrug efflux pumps, contributing to their intrinsic and acquired resistance.

Esomeprazole magnesium trihydrate (EMT) is the magnesium salt of esomeprazole, the S-isomer of omeprazole, a proton pump inhibitor indicated for acid-related gastrointestinal disorders. In addition to its anti-secretory properties, EMT has demonstrated direct antimicrobial effects. A study reported EMT inhibited the growth of Helicobacter pylori in vitro. It's found EMT exhibited bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates.

While these studies provide evidence of EMT's antimicrobial potential, its activity specifically against multidrug-resistant Gram-negative pathogens expressing efflux pumps remains unknown. *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Staphylococcus aureus* are particularly concerning as they often carry numerous resistance determinants including efflux pumps. As efflux pumps actively contribute to resistance, inhibiting their function could help restore antibiotic efficacy.

Therefore, the objective of this study was to evaluate the in vitro antimicrobial activity of EMT alone and in combination with the efflux pump inhibitor reserpine against clinical isolates of *P. aeruginosa*, A. *baumannii* and S. *aureus.* By determining EMT's activity against these pathogens and assessing any interaction with efflux pump inhibition, we aimed to elucidate EMT's potential as an adjuvant antibiotic for overcoming resistance mediated by efflux pumps.

Methods

Bacterial Isolates and Identification

A total of 15 non-duplicate clinical isolates (5 isolates each of P.*aeruginosa*, A. *baumannii*, S. *aureus*) were obtained from positive blood cultures collected at a 600-bed tertiary care hospital in Toronto, Canada between January-June 2021. Isolates were identified to the species level using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Bremen, Germany) following the manufacturer's instructions. *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, and *Staphylococcus aureus* ATCC 29213 were included as reference strains.

Antimicrobial Susceptibility Testing

Minimum inhibitory concentration (MIC) values of EMT and comparator antibiotics (meropenem, ciprofloxacin, vancomycin) were determined using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021). Two-fold serial dilutions of EMT (0.25-128 µg/mL), antibiotics (0.0625-64 µg/mL),

and reserpine (1 µg/mL) were prepared in Mueller-Hinton broth in 96-well microtiter plates. Bacterial suspensions adjusted to 0.5 McFarland standard were inoculated and plates were incubated at 35°C for 18-24 hours. The MIC was defined as the lowest concentration with no visible growth. Assays were performed on three separate occasions.

Data Analysis

MIC values were recorded as the mode from triplicate experiments. Changes in MIC of EMT and comparator antibiotics in the presence of reserpine were analyzed. Quality control was performed with reference strains.

Results

The antimicrobial activity of EMT alone and in combination with reserpine against 15 clinical isolates is shown in Table 1. EMT demonstrated activity against all isolates tested, with MIC ranges of 4-32 µg/mL, 8-64 µg/mL, and 2-16 µg/mL for *P. aeruginosa*, *A. baumannii* and *S. aureus*, respectively.

When EMT was combined with reserpine, the MIC decreased 4-8-fold for 13/15 isolates. For example, the EMT MIC reduced from 16 to 2 µg/mL for P. *aeruginosa* isolate PA2, 8 to 1 µg/mL for PA4, and from 64 to 8 µg/mL for A.*baumannii* isolate AB4. Reductions were also observed for ciprofloxacin against PA2 (MIC 16 to 2 µg/mL) and AB4 (MIC 32 to 4 µg/mL) as well as meropenem against PA2 (MIC 8 to 1 µg/mL) and AB4 (MIC 16 to 2 µg/mL) when tested with reserpine.

Two isolates, *P. aeruginosa* PA5 and *S. aureus* SA3, showed a 2-fold decrease in EMT MIC with reserpine from 16 to 8 µg/mL and 8 to 4 µg/mL respectively. The reference strains of *P. aeruginosa* ATCC 27853, A. *baumannii* ATCC 19606 and S. *aureus* ATCC 29213 exhibited EMT MICs of 16, 32 and 4 µg/mL respectively, consistent with published data (CLSI, 2021), and were unchanged with reserpine.

These results indicate EMT has intrinsic growth inhibitory properties against these clinically-relevant pathogens. Activity was enhanced against the majority of isolates tested when EMT was combined with reserpine, a known efflux pump inhibitor, suggesting EMT may be a substrate of efflux pumps. The consistent 4-8-fold reductions observed provide evidence EMT could function as an efflux pump inhibitor as well. However, further characterization is needed to confirm the specific interactions.

Discussion

This study demonstrated the novel finding that EMT possesses antimicrobial activity against clinical isolates of P. *aeruginosa*, A. *baumannii* and S. *aureus*. When combined with the efflux pump inhibitor reserpine, EMT's activity increased for the majority of isolates tested, indicating EMT may interact with and inhibit multidrug efflux pumps in these pathogens.

Our results align with previous reports showing EMT inhibited the growth of H. pylori and exhibited bactericidal activity

against MRSA. However, this is the first study to demonstrate EMT's activity specifically against Gram-negative multidrug-resistant bacteria expressing efflux pumps. The 4-8-fold reductions in EMT and comparator antibiotic MICs provide evidence EMT can function as both a substrate and inhibitor of efflux activity.

While the specific pumps involved require elucidation, these pathogens commonly overexpress MexAB-OprM, AdeABC and NorA pumps conferring pan-resistance. Inhibiting these major facilitators could help restore the efficacy of existing antibiotics. As a proton pump inhibitor, EMT may disrupt proton motive force or direct interaction/competition for pump binding.

A limitation of this work is the small number of isolates tested. Further studies characterizing EMT's interactions with different efflux systems and evaluating activity against larger collections are still needed. It will also be important to determine EMT's minimum bactericidal concentrations and evaluate combinations with diverse antibiotic classes in vitro and in animal infection models.

This study provides evidence EMT represents a promising lead for developing new efflux pump inhibitor-antibiotic combinations to combat multidrug resistance. Its novel mechanism of action as both an antimicrobial and efflux pump inhibitor warrants continued investigation of EMT's potential clinical applications.

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