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Efflux Pump Inhibitors and the Future of Antimicrobial Therapy

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ABSTRACT:

We are in a post-antibiotic era where once treatable bacterial infections are a renewed and serious threat to public health. This is largely driven by the overuse and misuse of antibiotics which is accelerating the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan drug-resistant (PDR) pathogens. One of the key resistance mechanisms in these organisms like *Acinetobacter baumannii* is the use of efflux pumps. These membrane proteins help bacteria survive by expelling a wide variety of antimicrobial compounds, which lowers intracellular drug concentrations. With slow antibiotic development, Efflux Pump Inhibitors (EPIs) have emerged as a solution to re-establish the activity of current antibiotics. EPIs work through different mechanisms: direct inhibition of pump activity, disruption of energy sources like the proton motive force, suppression of gene expression and interference with pump structure. Sources of EPIs are plant derived compounds (e.g. alkaloids like reserpine), microbial metabolites and synthetic molecules. EPIs are a cost effective and innovative adjunct to antimicrobial therapy which can reverse bacterial resistance, increase the antibiotic potency and inhibit efflux induced biofilm formation. While there are challenges like limited cell permeability, toxicity concerns and lack of clinical data. Development of EPIs is essential to preserve the utility of current antibiotics especially against high priority ESKAPE pathogens.

Introduction

The discovery of penicillin and streptomycin in the early 20th century marked the beginning of the antibiotic era, providing effective treatments for bacterial that bacteria have evolved and selected to health crisis where existing antibiotics are no longer working and the pipeline for new ones is dry. And to make matters worse,

infections that were once fatal. The mid-20th century was a bumper crop, with about half of the antibiotics we use today coming from that period. But we've used these drugs so widely and so carelessly be resistant to them. We're now in a global we're using broad spectrum antibiotics in animal agriculture. Multiple drug resistant (MDR), extensive drug resistant (XDR) and



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pan drug resistant (PDR) microorganisms have pushed us into a “post antibiotic era” where routine infections could once again be untreatable and deadly. The UN General Assembly has established a global framework to coordinate international action on antimicrobial resistance. Bacterial resistance mechanisms are many, including altered cellular permeability, modification of antibiotic targets, enzymatic inactivation of drugs and expression of efflux pumps. Efflux pumps are specialized bacterial transport proteins that actively pump antibiotics out of the cell, reducing intracellular drug concentration and conferring resistance. With no new antibiotics in the pipeline, targeting these efflux systems with efflux pump inhibitors (EPIs) is a promising way to restore existing antibiotics against resistant bacterial pathogens. (1) Antibiotic resistance is a global health crisis and MDR bacteria is a big part of that. One of the main defence mechanisms is the widespread expression of efflux pumps by bacteria. Bacterial cells use specialized membrane-bound proteins to expel various antimicrobial compounds, including antibiotics, helping them resist treatment. By minimising the intracellular saturation of these drugs, efflux pumps allow MDR bacteria to survive and thrive even in the presence of otherwise lethal antibiotic levels. *Acinetobacter baumannii* is a great example of a bug with multiple efflux pump systems and is a major public health threat. This Gram negative *coccobacillus* is a major cause of nosocomial infections and has been on the priority list of major health rotation of the efflux pump. (3)

Classification of efflux pumps

Efflux pumps are everywhere and are categorized into 6 families based on their

organizations including CDC, NIH and WHO due to its ability to acquire and transfer resistance genes. The global rise of carbapenem resistant *A. Baumannii* (CRAB) is alarming as carbapenems are the last resort antibiotics for severe hospital acquired infections.

With this growing threat EPIs are emerging as an option. EPIs are molecules that counteract the activity of bacterial efflux pumps. Their aim is to make resistant pathogens susceptible to conventional antibiotics so that antibiotic treatment can work. These inhibitors can work through competitive or noncompetitive binding to the pumps, dissipate the proton motive force required for pump function, suppress the gene expression of efflux pump components or interfere with their assembly. The global focus on *A. Baumannii* efflux pumps highlights the need for EPIs. These inhibitors can extend the life of existing antibiotics and give us new ways to handle life threatening, multiple drug resistant diseases. (2)

Efflux pump overview**Structure and function**

Knowledge of the structure and function of efflux pumps has been key to designing EPIs. For example, studies of AcrB's structure found clear spots where drugs attach and showed it uses a rotating motion to push them out of the cell. EPIs like ABI-PP bind tightly to a specific narrow hydrophobic region, an inhibitor binding pit, which branches off from the Distal Binding Pocket (DBP) in the pump. This binding is thought to malfunction the structure and energy source. Understanding each family is key to developing resistance therapies. These families are:

- ATP-binding cassette (ABC) transporters: Membrane bound pumps

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which utilise ATP hydrolysis to transport substrate. 2 transmembrane and 2 nucleotide binding domains, they confer resistance. PatAB is present in Gram positive bacteria manage the class fluoroquinolones, MacB present in Gram negative bacteria often forms tripartite structures to expel macrolides.

- Major facilitator superfamily (MFS) transporters: These are the largest and most diverse group, MFS proteins are uniporters, symporters or antiporters, they primarily harness the proton motive force (PMF). Antiporters contribute most to clinical resistance. NorA in *Staphylococcus aureus* exports fluoroquinolones and is linked to biofilm formation.
- Multidrug and toxin extrusion (MATE) transporters: These pumps use H⁺ and/or Na⁺ electrochemical gradients to extrude cationic compounds like norfloxacin. 12 transmembrane helices, NorM from *Vibrio cholerae* is a key example, uses both proton and sodium gradients.
- Small multidrug resistance (SMR) transporters: Small proteins with 4 transmembrane helices, SMR pumps form homo- or heterodimers and use the PMF. They confer resistance to lipophilic compounds, quaternary ammonium compounds. *E. Coli* EmrE is an example of this family.
- Proteobacterial antimicrobial compound efflux (PACE) transporters: Like SMR, these new pumps are resistant to biocides and pesticides. increasingly resistant pathogens.

Mechanism of Action

Efflux pumps are a major mechanism of bacterial antibiotic resistance and lead to a multidrug resistance phenotype by

AceI in *Acinetobacter baumannii* exports antiseptics like chlorhexidine.

- Resistance–nodulation–cell division (RND) transporters: The most clinically relevant in Gram-negative bacteria, RND systems are often overexpressed and give broad MDR phenotypes. These trimers form large tripartite structures across the cell envelope and use the PMF to remove a large range of substrates which involves many antibiotics. Examples are *E. Coli*'s AcrAB–TolC and *Pseudomonas aeruginosa*'s MexAB–OprM. RND systems also play a role in bacterial virulence, quorum sensing and biofilm formation. (4-10)

Defination, need and Mechanisms of efflux pumps

Efflux pump inhibitors (EPIs) are proteins that halt bacterial antibiotic resistance by blocking efflux pumps. These bacterial membrane transporters pump out various substances including antibiotics from the cell's interior, reducing the drug's concentration inside the cell and allowing the bacteria to survive otherwise lethal doses.

The Need for Efflux Pump Inhibitors

The problem of antimicrobial resistance (AMR) is a global health crisis, leading to longer hospital stays, expensive treatments and higher mortality rates. Over the last few decades the pace of new antibiotic discovery has slowed to a crawl and we are running out of options against pumping out or expelling a wide range of materials including different classes of antibiotics. MDR bacterial infections are often untreatable. So EPIs are seen as potential therapeutic agents that can revive the activity of existing antibiotics by



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blocking their efflux, thus increasing their concentration inside the bacterial cells. Antibiotics with EPIs have shown promising results in lab and clinical settings.

How EPIs work

1. Competitive Inhibition: EPIs bind to the substrate binding sites of the efflux pumps and prevent antibiotics from binding and being pumped out. PaßN is an example of a competitive inhibitor, it is a substrate itself for RND pumps.
2. Non-Competitive Inhibition: These EPIs bind to other sites on the efflux pumps and cause conformational changes that prevent the pump from pumping substrates. NMP causes a conformational change in AcrB and inhibits it non-competitively. Energy Source Blocking: Certain EPIs prevent the energy sources needed to run efflux pumps from functioning properly. CCCP suppresses pump action and interferes with ATP production and the proton motive force. Columbamine also disrupts proton electrochemical gradients and ATP production.
3. Downregulating Gene Expression: Genes encoding the efflux pumps may be inhibited by certain EPIs. It has been demonstrated that flavonoids such as curcumin and silybin downregulate these efflux genes.
4. Adjusting Membrane Permeability: Some EPIs, especially synthetic and plant derived compounds, can increase
3. Polyphenols: Epigallocatechin gallate is another in this category that enhances the activity of antibiotics such as tetracycline, erythromycin and ciprofloxacin against Staphylococci overexpressing TetK and against

membrane permeability. More antibiotics can get in, but too much permeabilization can be cytotoxic. (11-16)

Classification

EPIs are divided into two categories according on their origins: natural and synthetic.

Natural sources have historically produced a large number of novel medications, including variants of extensively researched antibiotics.

Naturally Derived EPIs

1. Plant-derived EPIs: Research has demonstrated that phytochemicals produced from therapeutic plants have the ability to block the efflux pumps of both Gram-positive and Gram-negative bacteria. This is important as it can restore the activity of antibiotics by allowing them to reach and maintain sufficient concentrations inside the bacterial cells (17-19).
2. Alkaloids: One example is the indole alkaloid reserpine, which is extracted from the roots of the plants Rauwolfia vomitoria and Rauwolfia serpentina. Reserpine has been used historically as an antipsychotic, however it is also an EPI against both MFS and RND efflux pump families (20). It has been shown to bind directly to the membrane protein (BMR) pump at the valine and phenylalanine residues, thereby blocking tetracycline efflux in Gram-positive B. subtilis. (21). Campylobacter species. However, safety concerns led to the discontinuation of this compound's further preclinical development (22).
4. Microbial-derived EPIs: Usually discovered by screening fermentation



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products, microorganisms contain a smaller percentage of EPIs EA-371 α and EA-371d, which were isolated from the fermentation of *Streptomyces* species, are two examples. These substances are crucial for preventing tripartite efflux pumps in multidrug-resistant Gram-negative bacteria and are known to be strong inhibitors of the MexAB-OprM pump in *P. aeruginosa* (23).

Synthetically Derived EPIs

1. Pyranopyridine derivatives: MBX2319 has no activity against *P. aeruginosa* but is a strong inhibitor of the AcrAB pump in *E. coli* (25,26). This compound enhances the activity of antibiotics that are substrates of the AcrAB pump (26).
2. Pyridopyrimidine derivatives: Both in vitro and in vivo studies have demonstrated the efficacy of pyridopyrimidine derivatives D13-9001 and D2 against the MexAB-OprM pump in *P. aeruginosa* (24,25).

Mechanism of Action

EPIs work by stopping efflux pumps in multiple ways and reducing antibacterial resistance. The main reason of blocking these pumps is to raise the intracellular concentration of an antibiotic and restore its activity in resistant bacteria. EPIs achieve this inhibition by interfering with the regulatory pathways that are needed for the expression of efflux pump genes. Chemically altering the antibiotic itself to prevent it from being recognized and pumped out by the efflux pump. Disrupting the assembly of the various parts that make up the efflux pump. Inhibiting the binding of the substrate (antibiotic) to the pump through competitive or non-competitive interactions with other molecules. Many studies have shown that combining EPIs with antibiotics increases antibacterial activity and prevents the drugs from being pumped out by efflux pumps. EPIs can also prevent efflux pump-mediated biofilm formation and reduce the antibiotic resistance of pre-existing biofilms.

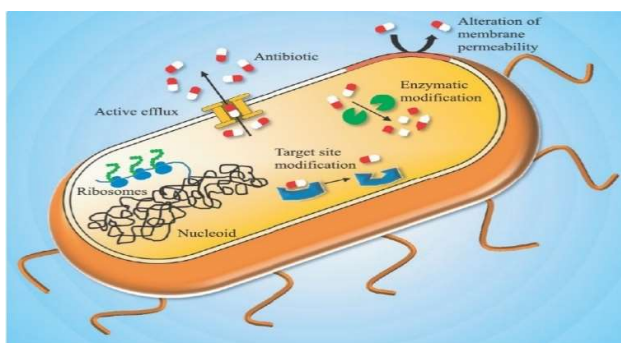


Figure 1: Mechanism of Action

Therapeutic Uses

EPIs are a big deal in the current antibacterial resistance landscape by reviving existing antibiotics. They can be used as a therapeutic to reverse antibiotic

resistance which is a cost effective solution. This removes the need for the drawn-out, costly, and time-consuming process of creating new antibiotics. Instead it allows doctors to use the established

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pharmacology of current antibiotics. The biggest implication of EPIs as therapeutic agents is they can reverse antibiotic resistance. In short, combining an antibiotic with an EPI is not only good for treating bacteria that are currently resistant but also a buffer against future resistance. Multidrug resistance (MDR) has become a major societal problem mainly due to the increasing antibiotic resistance across all organisms and the misuse and abuse of drugs which can lead to an antibiotic resistance pandemic. In this dire scenario EPIs are the light at the end of the tunnel by preventing drug escape from bacterial cells. Despite the challenges in developing them, the effectiveness of EPIs as therapeutic agents cannot be overstated. Their most impressive feature is they can restore the function of a drug to which bacteria have already developed resistance thus bypassing the need to create new drugs. The synergistic effect when used with other drugs has been shown. So using EPIs is a promising way to reduce bacterial antibiotic resistance in this era of MDR. Looking forward EPIs will be the savior of ESKAPE pathogens.

Challenges

Despite their promise, efflux pump inhibitors have several challenges.

Synthesis Issues: Efflux pump inhibitors from natural sources have complex and large molecules which makes synthesis difficult and resource intensive.

Problems with Synthetic EPIs: Although synthetic EPIs are typically simpler to manufacture than their natural counterparts, they do have certain drawbacks.

They may not be able to enter the body and function as planned because of their restricted solubility, high toxicity, and decreased cell permeability.

Lack of Data: An important challenge is the lack of preclinical and clinical data for EPIs.

Pharmacokinetic Considerations: To maximize its effectiveness, EPIs are typically administered in conjunction with antibiotics. For optimal pharmacodynamic effect, an EPI's pharmacokinetics should be matched to that of its antibiotic partner, although they don't have to be the same. The pharmacological activity of competitive inhibitors is likely determined by the point at which the body concentration of the EPI exceeds the concentration associated with the IC₅₀ of pump inhibition.

Future Directions

Efflux pump inhibitors offer a lot of hope in this era of antibacterial resistance by reviving existing antibiotics. They can reverse antibiotic resistance as a therapeutic agent and is cost effective as you don't need to develop new antibiotics – a time consuming, expensive and laborious process. You can also leverage the established pharmacological properties of existing antibiotics. The biggest implication is they can reverse resistance and be a buffer against future resistance. In the face of the social issue of multidrug resistance (MDR), EPIs serve as a "beacon of hope" by preventing drug escape. Despite the challenges, the effectiveness of EPIs as therapeutic agent cannot be overstated. Their best feature is to restore the function of a drug to which bacteria has already developed resistance so you don't need to create new medicines. The synergy of EPIs with other drugs have been shown. So using EPIs is a good strategy to reduce bacterial antibiotic resistance in this MDR era. In the future, EPIs will save lives by stopping ESKAPE germs from escaping.

**MANUSCRIPT ID: BTS0726003****Conclusion**

In summary, efflux pump inhibitors (EPIs) are a great way to combat antibiotic resistance especially against multi drug resistant bacteria. They are efficient against resistant bacteria because they directly block the bacterial efflux pumps, which raises the intracellular concentration of antibiotics. EPIs are classified based on their origin, natural complex compounds from plants and microbes and synthetic molecules, each with its own examples and mechanisms of action. The therapeutic potential of EPIs is huge, they can reverse existing resistance and most importantly provide a buffer against future resistance. This is scientifically sound and cost effective as it avoids the need to develop a new class of antibiotics.

Despite the challenges of synthesis, permeability, toxicity and lack of preclinical and clinical data EPIs are crucial in reviving current antibiotics and saving lives against ESKAPE pathogens. The synergy of EPIs with other drugs only highlights their importance in the global antibiotic crisis. We must continue to develop EPIs to ensure antimicrobial treatments work in the future.

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