

Efflux pump inhibitors: new hope in microbial multi-drug resistance

Eknath D. Ahire, Sanjay J. Kshirsagar

Department of Pharmaceutics, MET's Institute of Pharmacy, Affiliated to Savitribai Phule Pune University, Maharashtra 422003, India

ABSTRACT

From the last some decades, microbial multidrug resistance (MDR) has developed as one of the main treatments in many drug therapies. Due to this effect so, many sectors have been affected including the pharmaceutical sector, animal husbandry sector as well as the agriculture sector to some extent. There are so many mechanisms were developed by the microbes to generate resistance towards the medicines consisting of the development of degrading enzymes, structural modification in the microbes which is responsible to bind the drug, and last but not least the development of many efflux pumps to push drug molecules outside of the microbial cell. Many studies demonstrated that lots of antibiotics and anticancer agents are majorly get affected by efflux pumps present on the cell wall. These mechanisms have been observed in many common microbes including gram-positive and gram-negative microbes. As a result, efflux pumps should be taken on top priority for the minimization of multidrug resistance. Up to date, lots of work has been done regarding the permeation glycoprotein (P-gp) efflux pump inhibition, but still, there is a need to explore more innovative approaches towards MDR. In this current perspective, we have discussed the key points related to pre-existing and future prospects in this regard.

Key words: permeation glycoprotein, multidrug resistance, efflux pump, anti-microbial

INTRODUCTION

Microbial multidrug resistance develops when microbes terminate to persist defenseless to the medicaments which were formerly defenseless. The minimum inhibitory concentration (MIC), is the lowest concentration of an anti-microbial drug essential to

prevent microbial survival. In the era of 21st-century medical history, it has been encountered one of the darkest eras in front of the discovery of antibiotics. This was mainly due to the lack of available options for curing bacterial infections. Most Antibiotic drugs used so far have been discovered in the present era. At the beginning of the 1940s, antibiotic resistance concerning penicillin was reported

*Corresponding author:

Mr. Eknath D. Ahire

Department of Pharmaceutics, MET's Institute of Pharmacy, Affiliated to Savitribai Phule Pune University, Adgaon Nashik, Maharashtra 422003, India.

E-mail: eknathahire05@gmail.com

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for the first time in gram -ve bacteria. After the 1950s, the dosing, formulation, and administration methods of anti-microbial were expanded.^[1,2] Additionally, the mechanism of action of anti-microbial was re-investigated in detail. Nevertheless, meanwhile the start of the 1990s and the 2000s, anti-microbial resistance has increased to importance. The Centers for Disease Control and Prevention reported a mortality rate of 23,000 out of 2 million individuals in the United States of America (USA) who get diseased with antibiotic-resistant bacteria yearly. It has been showed that more often than not, the plasmids in a bacterial cell carry the expansion gene rather than the deoxyribonucleic acid (DNA). Over time, such resistant gene can be developed by the bacteria via progression or mutation and then be passed onto succeeding generations by duplication. An additional technique by which bacteria obtain resistant genes is horizontal gene transfer. Some antibiotic degrading enzymes such as different beta-lactamases destroy cephalosporin, penicillin, and other related class of anti-microbial that comprise beta-lactam rings.^[3-6]

Though, amongst the different multidrug resistance mechanisms, P-gp efflux pumps have been originate widely in gram -ve microbes and gram +ve microorganisms. Such extrusion of a structurally diverse class of antibiotics gives rise to microbial multidrug resistance (MDR) bacteria, i.e., bacteria resistant to more than one antibiotic drug. P-gp efflux pump is one of the major transporter solely responsible for the MDR effect in microbes. There are several p-gp efflux pump inhibitors that have been discovered to date but these conventional inhibitors have other issues too, such as secondary pharmacological and toxicological effects, accumulation in the different tissue mass that may cause toxic effects. In this current perspective, we have tried to explain the pre-existing problem of p-gp efflux pump and their current and future scope in this regard (Figure 1).^[7]

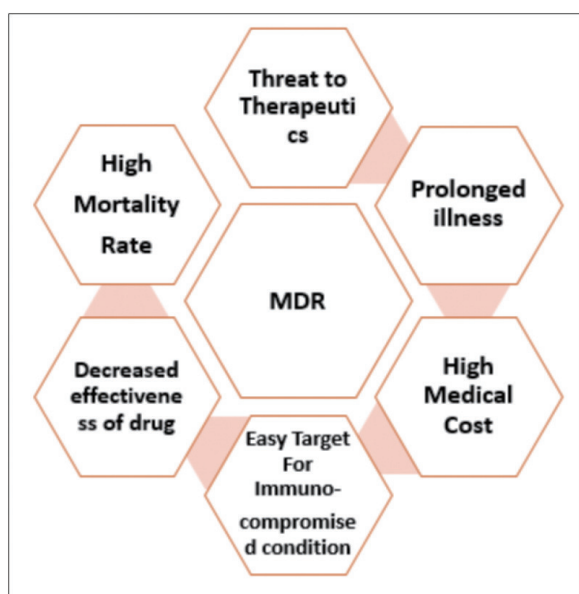


Figure 1. Consequences of multidrug resistance.

P-GP STRUCTURE AND PHYSIOLOGICAL ROLE IN THE BODY

Permeability glycoprotein also recognized as P-gp is MDR1. P-gp is one of the most important transporters in the cell membrane, majorly focused on the metabolism of the foreign particles and effluxing out all of them outside of the cell. P-gp efflux action is substrate-dependent and is reliant upon adenosine triphosphate (ATP). This protein is widely observed in the different microbes like fungi, animals, and bacteria, and is supposed to be expected convoluted in the protection mechanism in contradiction of foreign materials. P-gp is comprehensively dispersed widely in the body and contributes mainly in efflux roles of the intestine, bile ducts and liver cells, kidney cells (such as proximal tubules) and capillary endothelial, basically endothelial cells including blood-testis barrier, blood-brain barrier (BBB). This efflux transporter protein is also observed in other parts of the body such as pancreatic cells, adrenal gland, and colon. P-gp is mostly observed to be overexpressed in the cancer cells which inhibits cell entry of numerous anticancer agents hampering effective cancer treatment. It inhibits tissues from vulnerable noxious materials and improves the elimination of metabolites, but bile ducts secrete them in the lumen of the gastrointestinal tract (GIT).^[8,9]

P-gp exists in the human species in two isoforms as class I and class II. Class I isoform contains MDR1 ATP Binding Cassette Subfamily B Member 1 (ABCB1) transporter protein. Class II isoform contains MDR2 and ABCB4. The only gene P-gp transporter protein can transmit an extensive range of the chemical compounds which may be relatively dissimilar in their molecular weights in the case of different antibiotic compounds. P-gp transporter is recognized for efflux wide range of mechanically different compounds. Chemical compounds of the hydrophobic environment have a greater tendency to interact with anti-cytotoxic agents, steroids, cardiac glycosides, immune-suppressants, and many more.^[10,11]

P-gp is from the ATP Binding Cassette (ABCs) transporter subclass family, an ATP-dependent efflux pump with a molecular weight of 170 kDa, encoded by the human 207 MDR gene. It is made up of 12,801 amino acids arranged in a very precise form observed from 208 cDNAs. Structural properties show that P-gp has two symmetrical amino acids (N) and 209 carboxyl (C) ends in association to further ABC transporters. The efflux of 210 xenobiotics from the human body, is also contributing to various functions which involve almost 211 body functions including channel activity, from lymphocytes cytokine secretion, from adrenal glands steroid secretion, 212 dendritic cell migration, and cell death regulation beside 213 with participation in cell discrepancy.^[12] P-gp demonstrates expression for 5 dissimilar types of the genes comprising MDR1, MDR2, MDR3, 215 MDR1A, and MDR1B. Thus, MDR1 and MDR3 are known to be expressed in humans while MDR2, MDR1A, and MDR1B are observed expressions in other organisms. As MDR1 spreads widely in the body and is related to the MDR3 gene, there are nearly 217 drug molecules af-

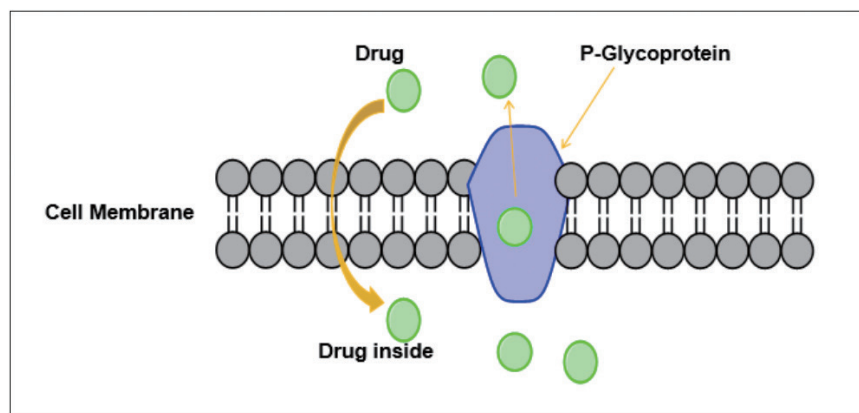


Figure 2. Schematic representation of P-gp structure and its inhibitory mechanism (Created with software ChemBioDraw Ultra 14.0. PerkinElmer, Waltham, Massachusetts, United States.)

fecting MDR1 (Figure 2).^[13]

P-GP EFFLUX PUMP INHIBITORS

Efflux pump inhibitors have been exposed to wide-ranging research for the checking of their mode of action. This has left many efflux pump inhibitors with proof of their action as a potentiator deprived of elucidating their action mechanism. In this case, the classification of efflux pump inhibitors grounded on their origin or sources becomes vital. Consequently, based on the source, EPIs can be classified into natural-based e.g. flavonoids, flavones, and chalcones Alkaloids Terpenes Polyphenols, etc. Secondly, it is synthetic source-based e.g. peptidomimetics, Quinoline and its derivatives, Pyranopyridine, pyridopyrimidine and their derivatives, Arylpiperidines, aryl piperazine, and their derivatives, etc.^[3] Other classes of efflux pump inhibitors are derived from microbes. Few are based on interfering with the driving force (energy generation) of the efflux pump. And some of the inhibitors with direct binding affinity to efflux pumps, and so on. Many natural types as well, as synthetic, conventional, and novel efflux pump inhibitors are discovered and studied, but still, there is no significant data is available to prove the exact mechanism and inhibition pathways behind the same. Therefore, there is a need to investigate novel inhibitors with ideal properties to restrict the MDR effect in microbes.^[3, 14, 15]

Classification of efflux P-gp pump inhibitors

Natural sources based efflux pump inhibitors

Flavonoids are secondary metabolites of plants with a polyphenolic structure. They were notion to promote health advantages due to their antioxidant action. Flavones are a subcategory of flavonoids with a non-saturated 3-carbon chain and double bonds between carbon-2 and carbon-3.^[16] The Hydroxyl group on the carbon-3 is lacking in flavones. Chalcones are absent from the carbon ring of usual flavonoid molecules. Therefore, they may be also known as open-chain flavonoids. It is present in the thyme leaves of the plant *Thymus vulgaris* and *Scutellaria baicalensis* georgi.^[17, 18]

Reserpine has been used as an antipsychotic and antihypertensive drug. It is isolated and extracted from the roots of the herb *Rauwolfia serpentina*. It has clutched interest as an auspicious efflux pump inhibitor that objectives for efflux pump inhibitions.^[19] Tests in numerous medical isolates, along with *Mycobacterium tuberculosis*, have proven potentiation in rifampicin pastime with the aid of using inhibiting anonymous efflux pump whilst used with piperine. In *Mycobacterium smegmatis*, piperine has been proven to reduce the MIC of ethidium bromide, representing its application as an efflux pump inhibitor throughout bacterial genera.^[20, 21] Testing numerous extracts of *Laminaria japonica* and *Sargassum horneri*, normally referred as brown algae and *Gracilaria* species, and *Porphyradenata*, and red algae, showed their ability as efflux pump inhibitors in opposition to drug-resistant *Escherichia coli*. Observations discovered that those extracts potentiated the activities of antibiotic drugs to various extents.^[6] The extracts and the drug clarithromycin showed synergism. The majority of the recognized efflux pump inhibitors have aromatic structures, while the extracts from numerous seaweeds integrated numerous terpenes, terpenoids, phenolic compounds, indoles, pyrrole derivatives, alkaloids, and halogenated aromatic compounds of their structures. Epicatechin gallate is barely greater efficacious than epigallocatechin gallate. However, at low concentrations, each compound had been mentioned to expose efflux mediation activities. The concept of wonderful binding sites of each of the molecules at the efflux pump with various affinities has been propounded. Small amounts of catechins enhance the efflux of the substrate through binding to excessive-affinity binding sites. Nevertheless, their characteristic as an efflux pump inhibitor is seen at high concentrations only.^[22, 23]

Synthetic sources based efflux pump inhibitors

Among 200,000 synthetic and herbal molecules screened, phenylalanine-arginine-beta-naphthylamide (PAβN), became the primary molecule recognized as an efflux pump inhibitor. With the aid of using RND efflux pump inhibition, PAβN restored gram-negative microorganisms' susceptibility to fluoroquinolones, macrolides, and chloramphenicol. Various PAβN analogs have been synthesized

after PA β N becomes unstable in biological systems including mice, rats, or human serum. Throughout their tests, they figure out the susceptibility potentiation of levofloxacin in opposition to *Pseudomonas aeruginosa*. It influenced stability in biological structures and additionally vulnerability potentiation of levofloxacin each in vitro and in vivo, just like the unique PA β N molecule. Numerous screening strategies in clinical traces of MDR microorganisms produced quinoline and quinoline-like molecules.^[17, 24] Quinoline and quinazoline derivatives represented an auspicious boom in drug liability to resistant clinical bacterial strains that overexpress efflux pumps some of the numerous derivatives. The MIC values of chloramphenicol, tetracycline, and fluoroquinolone have been depreciated 4-fold–16-fold whilst the above-mentioned derivatives have been administered. The search for novel EPIs resulted within the high-throughput screening of many N-heterocyclic molecules for his or her capacity to reverse MDR effects in bacterial strains.^[25] The spacer between the piperazine ring and the benzene ring and the presence of substituted halogens on the benzene ring became responsible for regulating the inhibitory activity of aryl piperazines. Analysis of Arylpiperazines used for MDR restoring pastime found out that the efficiency and efficacy have been more desirable because of the spacer's elongation and substituted halogens.^[26, 27]

Efflux pump inhibitors that interfere with the driving force

Bacterial cell substrates, efflux pump inhibitors, concentrates on strategies that produce energy have a good chance to weaken the efflux pump's utility. Progressions that produce energy consist of ATP hydrolysis, proton motive force, Na⁺ gradient, and in a few belongings, a combination of proton cause force and Na⁺ gradient. Similarly, MDR microorganisms had been determined to employ a couple of types of efflux pumps. Among the most popular laboratory efflux pump inhibitors, carbonyl cyanide-m-chlorophenylhydrazone (CCCP) stays the most popular ionophore.^[28, 29] It hinders the proton cause force by affecting the transmembrane capability and transmembrane pH. Accordingly, the bacterial cells are metabolically neutralized, it is thought whether the CCCP synergism with multiple antimicrobials is because of the efflux pump deactivation or the bacterial cells' metabolic deactivation.^[30] Apparently, CCCP renovated tetracycline activity in opposition to *Helicobacter pylori* and *Klebsiella* species. Supposedly, carbapenems and CCCP synergism is now no longer dependent on the inhibitory interest of CCCP. This most effective approach that helps the speculation referred to above of metabolic deactivation of bacterial cells via way of means of CCCP, ensuing in synergism thru antimicrobials. While, CCCP is constrained to the laboratory completely because of its toxicity in mammalian cells.^[31, 32]

Efflux pumps inhibitors with direct binding affinity

Additional mechanism with the aid of using efflux inhibition proceeds place is with the aid of using directly required to the efflux pump. The necessary conditions can be competitive, i.e., binding to the efflux pump's substrate-binding site such that the substrate-binding is absolutely obstructed.^[33] Another type of binding

may be non-competitive, i.e., binding to the efflux pump, thereby reducing its affinity in the substrate direction. However, inhibition by non-competitive binding may be effortlessly conquered with the aid of using microorganisms by efflux pump protein modification. PA β N, a substrate for Resistance-nodulation-division (RND)^[34] sort of efflux pumps, has been determined to potentiate the activity of levofloxacin, erythromycin, and chloramphenicol in opposition to *Pseudomonas aeruginosa* expressing efflux pumps by using competitively binding to the efflux pumps.^[35] PA β N now no longer potentiates the activity of tetracycline or carbenicillin. This shows the possibility that tetracycline and carbenicillin would possibly have exceptional binding sites than the ones of PA β N. Specific indole-derived EPIs bonded with the outer/exit duct of the TolC efflux pump expressed in *Escherichia coli*. By binding to the outer duct, efflux channel closing turned into achieved, and efflux was attenuated.^[36–38]

CURRENT STATUS OF EFFLUX PUMP INHIBITORS

Efflux pump inhibitors are particular chemical compounds that hamper antibiotic drug compounds' active transport exterior to the microbial cell wall.^[31] The approach behind the usage of efflux pump inhibitors is based on the idea that any compound responsible to enhance the intracellular concentration of active anti-microbial drug compounds will inhibit the resistance conferred to that microbial cell by efflux pumps. Other methods using this concept are currently employed such as i.) Enhanced cell wall penetration ii.) Drug transportation by passive transport. These methods target the developed resistance and the inherent properties of the bacteria. We can attain the p-gp efflux pump inhibition by different approaches, comprising, i) Counter-regulating the expression by hindering the genetic regulation, ii.) Manipulating the anti-microbial drugs undistinguishable (as substrates) by the efflux pump iii.) Obstructing the association of well-designed efflux pumps (Obstructing the translation process), iv) Blocking the pump to inhibit binding of anti-microbial compounds to the binding site, and v.) Obstructing the mechanism that produces the driving force of efflux pumps.^[16, 26, 29]

In the 1990s, R and D in the area of efflux pump inhibitor began. Widespread laboratory investigation has established that efflux pump inhibitors is one of the greatest hopeful prospects for combating increased anti-microbial resistance in microbes. Consequently, the expansion of efflux pump inhibitors has also been observed as a revolutionary invention.^[33] Nevertheless, technical, academic, administrative and commercial factors continue to challenge the commercialization and cost-effectiveness of efflux pump inhibitors. An important impediment is the value of efflux pump inhibitor as a pharmaceutical preparation. Pharmaceutical monsters abstain from being accompanied with this field since efflux pump inhibitors are technically a completely dissimilar and novel compound that in the pharmaceutical segment is considered as a New Chemical Entity (NCE). The noteworthy problems with NCEs is that their development, analysis, and modification will

involve wide-ranging experimentation. At last, these experiments will contribute to an increase in new documents or alterations to the old information. This means an changing or retaining presently known data is related to anti-microbial with a thoroughly-documented pharmacological outline and clinical data from plentiful patient records from both clinical trials and prescriptions. Development is a very monotonous task.^[25] Academicians regularly search for clinical trials from natural and synthetic bases. Though, their large-scale manufacturing has not yet been considered at the laboratory level. Efflux pump inhibitors from natural sources have a problematical and higher molecular weight. This creates them challenging to manufacture. On the other hand, Efflux pump inhibitors from synthetic bases are easy to prepare. But these molecules normally show toxicity, solubility, and cell permeability issues. The innovation of NCEs needs a broad investment in relations to equal founding and time. It is worth noting that hard work is mandatory to meet strict guidelines and standards. Since financially the profits are diffidently productive, this determines efflux pump inhibitors or other NCEs.^[18, 39] Therefore, there is a need to find out other natural-based as well as economic alternatives which can inhibit efflux pumps.^[17]

A FUTURE PROSPECT OF EFFLUX PUMP INHIBITORS

Nowadays, there is a need for pharmaceutical industries to invest in such projects which may solve the problem of MDR. Efflux pump inhibitors can also not be used alone or in combination with formulation. They will be used to increase the action of anti-microbial drugs, creating the utmost feasible combination treatment selection. Consequently, there should be no chemical interaction between efflux pump inhibitors and anti-microbial drugs. The pharmacokinetics and pharmacodynamics of both the anti-microbial compound and the efflux pump inhibitors must accompany each other for an effective therapeutic combination. Nevertheless, this knowledge gets extreme attention as far as their clinical investigation or application is troubled. One example is the combination of a calcium channel blocker (verapamil) with an anti-microbial drug (clarithromycin) has been witnessed to be dangerous. Clarithromycin aims at a cytochrome that facilitates the metabolism of verapamil. When these drugs are used in combination, accumulation of verapamil takes place efflux pump inhibitors have been witnessed to demonstrate specificity in their proficiency to potentiate the action of anti-microbial.^[25] This means that, in contrast to a s specific efflux pump, the efflux pump inhibitors that potentiate one anti-microbial drug's action do not potentiate other anti-microbial drugs' action for the similar efflux pump. Correspondingly, many efflux pump inhibitors target a definite substrate-binding site inside the efflux pump. Consequently, in order to confirm competitive inhibition, an expressively high efflux pump inhibitors concentration would be essential for the pump's preferred substrate anti-microbial interaction.^[40, 41]

Several approaches have been applied to prevent the efflux pump

of such drugs via efflux transporters, comprising combination with P-gp inhibitors, chemical alterations, and others, but with inadequate results. Excipients or inactive ingredients used in different formulations are purposefully added in various potent formulations to modify the properties of drugs. At the moment, there is growing attention in exploring the inhibitory action of these conventionally used excipients on the efflux pump, as they have been designated to origin delicate variations, which can disturb efflux pumps on the cell membrane. Numerous literatures have clarified the role of definite P-gp inhibitors in enhancing drug delivery, nevertheless, there is not abundant data to discover excipients for their characteristic meaning and P-gp inhibitory action. The excipients can be used to modify membrane transporter inhibition action and explore the mechanism involved. Definite formulations are formulated by means of such excipients or ingredients which may augment drug absorption and reduce associated toxicity. Therefore, the pharmaceutical R and D department need to invest in such novel projects.^[42, 43]

CONCLUSION

Microorganisms resistance to many antimicrobial drugs is increasing day by day and this concern has become one of the major challenges for existing anti-microbial drugs. This problem can't be solved by NCEs discovery, because NCEs found that sufficient money and time were needed. Therefore, efflux pump inhibitor seems to be a feasible way for MDR microorganisms. Though, these problems should by no means demoralize the profits efflux pump inhibitors give. The efflux pump inhibitors give a novel perspective on the use of existing anti-microbial drugs, preventing scientists and clinicians from spending a lot of time distracting themselves from discovering new anti-microbial drugs. Efflux pump inhibitors may have enough potential to make effective currently available anti-microbial drugs. Efflux pump inhibitors are encouraging since they have been shown to inverse MDR but have not been seen to build up MDR. Despite eye-catching approaches, efflux pump inhibitors practice has numerous breaches that must be shielded beforehand creating it accessible for the convention. The current era needs to solve the pre-existed efflux pump inhibitors problems and to be re-investigate the alternate molecules of efflux pump inhibitors, such as different formulation of excipients which may be able to reduce all efforts.

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Ahire ED wrote and revised the first draft. English editing and proofreading was performed by Kshirsagar SJ both authors are

agreed the final version and submitted the article.

Ethics approval and consent to participate

Not applicable.

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Conflicts of interest

Eknath D. Ahire is an Editorial Board Member of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and his research groups.

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