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Next-generation strategy for treating drug resistant bacteria: Antibiotic hybrids

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Resistance against nearly all antibiotics used clinically have been documented in bacteria. There is an ever-increasing danger caused by multidrug-resistant Gram-negative bacteria in both hospital and community settings. In Gram-negative bacteria, intrinsic resistance to currently available antibiotics is mainly due to overexpressed efflux pumps which are constitutively present and also presence of protective outer membrane. Combination therapy, *i.e.*, use of two or more antibiotics, was thought to be an effective strategy because it took advantage of the additive effects of multiple antimicrobial mechanisms, lower risk of resistance development and lower mortality and improved clinical outcome. However, none of the benefits were seen in *in vivo* studies. Antibiotic hybrids are being used to challenge the growing drug resistance threat and increase the usefulness of current antibiotic arsenal. Antibiotic hybrids are synthetic constructs of two molecules which are covalently linked. These could be two antibiotics or antibiotic with an adjuvant (efflux pump inhibitor, siderophore, *etc*.) which increases the access of the antibiotics to the target. The concepts, developments and challenges in the future use of antibiotic hybrids are discussed here. Majority of the studies have been conducted on fluoroquinolones and aminoglycosides molecules. The antibiotic tobramycin has the property to enhance the action of antimicrobial agents against which the multidrug-resistant Gram-negative bacteria were earlier resistant, and thus potentiating the action of legacy antibiotics. Antibiotic hybrids may have a role as the silver bullet in Gram-negative bacteria to overcome drug resistance as well as extend the spectrum of existing antibiotics

Key words Antibiotic hybrid - antibiotic resistance - Gram-negative bacteria - multidrug - resistance - quinolone

Introduction

The prospect of post-antibiotic era has become a dangerous reality due to rapid global spread of multidrug-resistant Gram-positive and Gram-negative bacterial pathogens in both hospital and community settings[1,2]. The various mechanisms of acquired resistance include mutation in drug target site, enzymatic degradation of antibiotics, active efflux through porins and other permeability barriers. Due to progressive selective pressure of separate and distinct groups of antibiotics, the transfer of gene responsible for drug resistance through plasmids, integrons and transposon has accelerated this spread[3]. However, the problem for Gram-negative bacteria is conceivably graver than Gram-positive pathogens due to these being more commonly multidrug-resistant (MDR)[3]. To counter the diminishing antibiotics available,

Infectious Disease Society of America launched its 10-20 initiative which was to develop, at least 10 new antimicrobial agents by 2020 to treat bacterial infections[4]. However, only six of these (ceftolozane-tazobactam, ceftaroline fosamil, ceftazidime-avibactam, meropenem-vaborbactam, delafloxacin and secnidazole) have been developed and found effective in the treatment of drug-resistant Gram-negative bacterial infections[5]. Therefore, the treatment of MDR Gram-negative bacterial infections remains a serious problem due to restricted availability of drugs. It is, therefore, vital to expand our antimicrobial arsenal or develop new remedial and restorative goal plan to overcome drug resistance in these organisms.

Challenges in construction of new antibiotics for Gram-negative bacteria

In Gram-negative bacteria, a thick, asymmetric outer membrane (OM) and inner membrane (IM) separate thin layer of peptidoglycan present in the periplasmic space. The OM is a bilayer with phospholipids forming the inner layer and an outer layer consisting of three zones. These are inner lipid A (hydrophobic layer), middle core oligosaccharides (hydrophilic layer), and outer O antigen forming lipopolysaccharides (LPS) (hydrophilic layer)[6]. Thus, the OM has limited membrane permeability to hydrophobic molecules which limits the passage and movement of these molecules across the OM and also due to lower OM fluidity because of systematic and well organized packing of lipid A[7]. Due to the structural differences and reorganization of LPS especially lipid A, there exists a variation in the rate of drug permeability among Gram-negative bacteria. An example of this is *Escherichia coli* having its OM permeability 12- to 100-fold higher as compared to *Pseudomonas aeruginosa*[8]. The IM barrier limits the action of antimicrobial agents with cytosolic targets. The phospholipid bilayer of IM restricts the diffusion of hydrophilic molecules[8]. Therefore, it is easier for hydrophobic molecules to cross the IM as compared to OM through passive diffusion. Due to these two protective lipid bilayers in Gram-negative bacteria, drugs with cytosolic targets have problems with permeability.

The charged or non-charged small hydrophilic molecules (β-lactams, fluoroquinolones and sulphonamides) enter into the periplasmic space through the non-specific protein channels called porins[9]. These barrel-shaped protein channels permit the water-soluble molecules to transverse through their water-filled cavity across the restrictive hydrophobic OM. However, only small molecules (≤600 g/mol) can pass through their narrow channels. Further, this porin-mediated uptake varies among Gram-negative bacteria[10]. For example, in *P. aeruginosa,* the OM protein F (Opr F) porin has less OM permeability than *E. coli*. This is due to structural conformation of Opr F porin that allows slow diffusion of solute than the classical porins present in *E. coli*[11]. In *P. aeruginosa* restrictive OM and porin-mediated influx results in low-intracellular drug concentrations, which are further aggravated by the presence of abundant multidrug efflux pumps. These pumps are membrane-bound efflux proteins that expel molecules from the periplasm outside the bacterial cell or from the cytosol into the periplasm[12].

It is, therefore, vital to manufacture new drugs or create novel remedial, restorative goal plans that can control and overcome drug resistance in these organisms. This has led researchers to explore innovative approaches to find an effective compound against multidrug-resistant bacteria (MDR) bacteria which does not develop resistance easily[13]. Hence, among the various strategies adopted to treat these resistant bacteria and overcome their resistance mechanism combination therapy was considered *i.e.*, to use antimicrobial agents with more than one inhibitory mechanism of action for the same treatment. The concomitant use of two or more antibiotics ensures coverage of all possible pathogens and decreases the chances of resistance development[14]. The choice of using two antibiotics or antibiotic with an adjuvant will depend on the bacteria causing infection and their known resistance profile. The main modes of action by which combination of two molecules enhance the activity of each other are: (*i*) in bacterial isolates where mutation within the target site caused resistance, another antibiotic is added to have a multitarget approach, (*ii*) preventing the degradation of antibiotic by enzymes, so as to achieve optimum antibiotic concentration, (*iii*) by inhibiting the efflux pump inhibitors (EPIs) the primary drug is retained inside the bacterial cells, and (*iv*) inhibiting the intrinsic repair mechanism of cells to the primary drug[15]. All of these strategies involve use of combinations of more than one antibiotic or of an antibiotic molecule with an adjuvant. However, treatment of Gram-negative MDR infections with combinations showed limited clinical benefit and this *in vitro* synergy testing did not translate into clinical benefits because of different pharmacokinetic properties of the drugs in combination[16].

Antibiotic hybrids

Antibiotic hybrids consist of two covalently linked pharmacophores with dissimilar mechanism of action[17]. This combinations of antibiotics, with either another antibiotic or with an adjuvant (which works to increase the approach and access to the target site or enhance the efficacy of primary antibiotics) are designed to overcome the existing resistance mechanism with either or both drugs[18]. The covalent link can be cleavable or non-cleavable. In case of cleavable link, the antibiotic hybrid is biotransformed at the site of action enzymatically, which constitutes a hybrid prodrug approach. In addition, the non-cleavable link remains unaffected in the body throughout time and action course[19,20]. Alternatively, two or more pharmacophores can be merged with the purpose of creating superior molecules. Therefore, a hybrid antibiotic is defined as a synthetic construct of two or more molecules or pharmacophores, developed with the aim to elicit a desired antimicrobial effect.

The proposed theory of antibiotic hybrids is that combination therapy suppresses drug resistance evolution better than monotherapy having a single-pharmacokinetic profile. It is hypothesized that one of the two therapeutic agents hybridized into a single molecule may convey extra benefits that were lacking in individual molecules. The antibiotic hybrid prodrug is cleaved into two functional molecules, each having its own drug metabolism and elimination whereas the antibiotic hybrid with the non-cleavable covalent bond behaves as a single molecule regarding metabolism and excretion during its presence in the body.

Conceptual challenges in designing antibiotic hybrids

The antibiotic hybrid against Gram-negative bacteria encounters many inherent complications. There occurs restricted cellular penetration of hybrid agents across both the membranes of Gram-negative bacteria for antibiotic hybrid having more than 600 g/mol of molecular mass. In addition, these high-molecular-mass antibiotic hybrid will also not transverse through non-selective porin channels[21]. Therefore, to overcome this permeability problem, antibiotic hybrid must be designed to utilize the porin-independent uptake mechanism of one or more of the parent constituents. For example, aminoglycoside class of antibiotics enter via a self-promoted OM uptake process, followed by energy-dependent IM uptake to access cytosol to produce its action[21]. An antibiotic hybrid which acts on non-intracellular targets will avoid the permeability issue. Another challenge lies in designing of the covalently linked two pharmacological agents together so that the connecting site and the physicochemical properties of the chosen linker will retain the functional integrity[21]. Since the last few years, many antibiotic hybrids have entered trials, but only a few have been reported to progress to clinical trials[17,18,20-22].

Quinolone/fluoroquinolone compounds

The most widely studied hybrid compounds contain the fluoroquinolone class of antibiotic linked to another antibacterial agent. The reasons why fluoroquinolones are broadly utilized are many: (*i*) fluoroquinolones target two enzymes inside the bacterial cell, topoisomerase IV and DNA gyrase, thus inhibiting DNA replication and transcription[23]. Therefore, if there is any steric interference imposed by a linker or a second partner leading to disturbance in the binding of one target, it might be compensated by targeting the other enzyme without a substantial reduction in antibacterial potential, (*ii*) another major benefit is the structure-activity relationship of fluoroquinolones *i.e.*, the basic amine group in the C-7 piperidino moiety as a suitable point for attachment of various bulky substituents, (*iii*) fluoroquinolone are stable under a wide variety of synthetic conditions making them ideal candidates for hybrid formation and development[24]. But due to their strong bactericidal activity, very low minimum inhibitory concentration (MIC) values and the spontaneous mutations of targeted enzymes, fast rates of resistance development have been reported for this type of antibiotics[24]. Various antibiotic hybrids have been developed using fluoroquinolones. A brief discussion about these prominent hybrids is given below:

(*i*) Cadazolid is a hybrid containing a quinolone molecule with an oxazolidinone. Cadazolid has poor solubility in aqueous solution due to its acidic and lipophilic nature. Its absorption from intestine is insignificant after oral dosage, so its systemic bioavailability is negligible, therefore, it is used in preclinical and clinical trial for treating *Clostridium difficile* infection (CDI)[25]. It has two-fold mechanism of action, strongly inhibiting protein synthesis and weakly inhibiting bacterial DNA synthesis. This synergy could be because of favourable physicochemical properties of fluoroquinolone portion which leads to easy bacterial cell permeation. In a study to investigate the mode of action of cadazolid using

a macromolecular labelling assay, it showed strong inhibition of protein and DNA synthesis inhibition in both quinolone and linezolid-resistant *C. difficile* isolates. In addition, it had greater potency than ciprofloxacin (64-fold), linezolid (8- to 64- fold) and moxifloxacin (8- to 64-fold). Cadazolid showed better activity to *C. difficile* strain in time-kill assays[26]. This is an emerging multidrug-resistant strain of *C. difficile* which is associated with multiple outbreaks having an enhanced toxin A and B production and high level fluoroquinolone resistance. Cadazolid showed faster 3 × $\log_{10}$ cfu reduction as compared to vancomycin within 24 h for this strain. In addition, cadazolid being a potent inhibitor of *C. difficile's* toxin and sporulation has further beneficial action. Unlike other antibiotics used in treating CDI, even at sub-growth inhibitory concentration of cadazolid (0.5 MIC) toxin production and sporulation were inhibited[27]. Furthermore, cadazolid has no adverse effect on normal intestinal microbiota; it is a better choice than other antibiotics. Cadazolid has lower propensity to develop resistance because it demonstrates lower resistance frequencies ($<10^{-10}$) and lack of cross-resistance with other antibiotics used to treat CDI. In addition, increase in MIC was minimal and after three selection steps[28]. In phase I clinical trial, cadazolid was evaluated to assess safety, acceptability and pharmacokinetics in 64 healthy male volunteers. No dose-limiting adverse effects were noted, only headache and diarrhoea were reported. In addition, cadazolid was mostly (81-93.5%) excreted by faecal route. Since kidney and liver have negligible role its excretion; it is non-toxic in patients with compromised hepatic and renal functions[28]. In phase II trial, done to study efficacy, safety and tolerability of cadazolid versus vancomycin in three treatment group of CDI patients cadazolid was found to be comparable or superior to vancomycin in clinical cure rate with lower reoccurrence rate[29]. Currently, two phase III clinical trials are underway with expected 1280 patients to be enrolled[30]. Further, study needs to be done to determine its clinical utility, teratogenic potential and absorption from inflamed intestinal area[30].

(*ii*) MCB-3681 is a hybrid antibiotics combining oxazolidinone and fluoroquinolone pharmacophores[18]. MCB-3681 and cadazolid show strong structural similarity. It has good *in vitro* antimicrobial activity against a range of multi-resistant Gram-positive organisms such as methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *Enterococcus*, *E. faecalis* (resistant to linezolid and ciprofloxacin) and *E. faecium* (resistant to linezolid and ciprofloxacin). In addition, MCB-3681 has shown to overcome resistance to either/both of its constituent parts[18]. Recently, MCB-3681 showed good *in vitro* activity against *C. difficile*. MCB-3681 demonstrated lower MIC to *C. difficile* strains as compared to cadazolid, linezolid and ciprofloxacin, but did not have any effect on *C. difficile* toxin production or spore production. The influence of MCB-3681 on human microbiota showed no abnormalities[31].

(*iii*) CBR-2092, a multivalent antibiotic is a hybrid of rifampicin pharmacophore with 4H-4-oxoquinolizine sub-series of fluoroquinolone antibiotics[32]. This dual action hybrid has demonstrated many benefits over rifampin and fluoroquinolone combination treatment. CBR-2092 showed potent activity against RNA polymerase and an inhibitor of topoisomerase IV and DNA gyrase, and it had low susceptibility for resistance development because it was not a substrate for fluoroquinolone efflux mechanisms. CBR-2092 exhibits stronger activity than one or both parent drugs. Since CBR-2092 is an inhibitor of three essential bacterial enzymes, the frequency of single-step resistance against wild-type *S. aureus* would be exceptionally low *i.e.*, $<10^{-2}$. The equivalent values for rifampicin and ciprofloxacin were 2.9×10^{-8} and 5×10^{-9}, respectively[33].

(*iv*) Neomycin B combined with ciprofloxacin forms a potent hybrid which exhibits good activity against Gram-positive and Gram-negative bacteria and also delays development of drug resistance. This neomycin B-ciprofloxacin hybrid showed activity against the MRSA, MDR *E. coli* and Gram-positive organism *Bacillus subtilis*[34]. Two main hybrids based on the different linkers structure are described - hybrid 8 and hybrid 9, which differ in the ideal spatial length and physicochemical property desired for optimum action. The neomycin B-ciprofloxacin hybrids has enhanced antibacterial activity as compared to neomycin B but not ciprofloxacin. Hybrid 8 showed higher *in vitro* inhibitory activities of DNA gyrase (15-fold) and topoisomerase IV (20-fold) than ciprofloxacin. However, this high degree of *in vitro* inhibition of DNA synthesis for hybrid 8 did not relate with

MIC values[34]. This could be because the hybrid has a high-molecular-mass, which curtails its ability to reach optimal intracellular level as compared to ciprofloxacin which reaches through porins (OM) as well as passively diffuses (IM)[34]. Even after 15 serial passages at sub-inhibitory MIC concentration hybrid 8 did not develop drug resistance for *E. coli* and *Bacillus subtilis*[34]. It was also found that MIC against *E. coli* increased for ciprofloxacin, neomycin B and neomycin-ciprofloxacin hybrid by 75-fold, 4-fold and 20-fold, respectively[34]. In the neomycin-ciprofloxacin hybrid, the main antibacterial activity was mediated by ciprofloxacin pharmacophore whereas delay the emergence of drug resistance was due to the neomycin B pharmacophore[35].

(*v*) A novel pharmacophore linking S tyrosyl-tRNA synthetase inhibitor covalently to 7th position of fluoroquinolone has been developed. These hybrid antibiotics possess broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. The mechanism of action is inhibition of both DNA gyrase and tyrosyl-tRNA synthetase[36]. This antibiotic hybrid composed of 3-(2-fluorophenyl) furan-2(5H)-one, is a potent inhibitor of bacterial protein synthesis. This hybrid displayed a potent action and was 30- to 50-fold more effective than ciprofloxacin against MRSA, MDR *E. coli* and tetracycline-resistant *B. subtilis* and had MICs <1 μg/ml against these three strains[36].

(*vi*) A hybrid antibiotic combining ciprofloxacin with pyrazinamide has been developed with broad-spectrum antibacterial activity against non-replicating persistent *Mycobacterium tuberculosis* (Mtb). Despite the compound having good pharmacodynamic and pharmacokinetic property, it was inactive against DNA-gyrase[37]. In addition, potent action against fluoroquinolone-resistant Mtb strains was demonstrated, but further studies should be done to look for development of resistance and toxicity[37].

(*vii*) A hybrid containing two fluoroquinolones with novel bacterial Type-II-topoisomerase inhibitors (NBTIs) has been developed, both antibiotics target GyrA. This hybrid has the advantage of overcoming drug resistance due to single point mutation as both the molecules target the same enzymes[38]. This shows activity against MRSA, *Klebsiella pneumoniae* and *Acinetobacter baumanii*. In addition, NBTI has retained activity against quinolone-resistant strains[38].

Tobramycin-based hybrids as adjuvants-potentiates legacy antibiotics

In 2017, carbapenem-resistant *P. aeruginosa* was ranked as a critical (priority 1) pathogen[39]. *P. aeruginosa* causes most of nosocomial infections in immunocompromised patients, and its treatment is quickly becoming elusive. One of the main reasons is that its impermeable OM is a major impediment that antibiotics face resulting in low penetration and uptake. Tobramycin-based hybrid was proved to be effective because of many reasons: (*i*) Since tobramycin enters the cytosol by self-promoted uptake, this can benefit transport of second hybridized antibiotic inside the cell, (*ii*) aminoglycosides, at low concentration, inhibit protein translation by interacting at rRNA level and at higher concentration disrupt the bacterial membrane (known as pleiotropic mechanism of antibacterial action)[40,41], (*iii*) tobramycin-containing hybrids have intrinsic physicochemical attributes that cause "resuscitating" the potency of antibiotics against MDR bacteria, especially *P. aeruginosa* against which there was earlier resistance[42]. The proposed mechanism of action is that the tobramycin-ciprofloxacin hybrid perturbs the OM of *P. aeruginosa* in a dose-dependent manner. Most common mechanism of resistance to fluoroquinolones in *P. aeruginosa* is due to overexpression of multidrug efflux pumps, therefore, the hybrid would compromise the efficacy of efflux pump[43]. This also leads to influx and bioaccumulation of various molecules which usually do not transverse the OM such as vancomycin, erythromycin, rifampin and novobiocin. It was found that amphiphilic aminoglycosides *i.e.*, tobramycin attached to aliphatic hydrocarbons have beneficial immunomodulatory functions. These tobramycin analogues selectively induce the chemokine interleukin (IL)-8 in macrophages. Since IL-8 is a potent neutrophil chemotactic factor, which causes the migration and activation of monocytes, lymphocytes, basophils and eosinophils leading to the resolution of infections. This may be exploited therapeutically[43-45].

Since tobramycin produces more than one effect (pleiotropic action) and because of its self-promoted uptake mechanism, various antibiotic hybrids have been developed with tobramycin and the choice of second molecule attached to this hybrid's scaffold varied. A few tobramycin antibiotics hybrids are discussed below:

(*i*) Tobramycin-moxifloxacin hybrid: moxifloxacin was selected in making the antibiotics hybrid

because of its bulky structure it was less affected by efflux mechanism and was robust enough to withstand chemical manipulation. This hybrid demonstrated potent antipseudomonal activity against MDR *P. aeruginosa*, extremely drug resistant (XDR) *P. aeruginosa* (resistant to cephalosporins, carbapenems, fluoroquinolones and aminoglycosides but susceptible to colistin) and pandrug-resistant (XDR and resistant to colistin) *P. aeruginosa* isolates, and in addition, there was low likelihood of resistance development[42]. The mechanism of action of tobramycin-moxifloxacin hybrid core structure was that it enhanced OM permeability and reduced efflux by dissipating the proton motive force of the cytoplasmic membrane in *P. aeruginosa*[43].

(*ii*) Tobramycin-lysine peptoid hybrid: A new class of antibiotic hybrid links tobramycin to lysine conjugates, containing an amphiphilic tobramycin-C12 linker. This hybrid retains the sensitivity of Gram-negative bacteria to legacy antibiotics. Similar to other tobramycin hybrids, this hybrid improves OM cell penetration to various molecules and dissipates the proton motive force, whose function is to energize efflux pumps. These conjugates overcame resistance to rifampicin and minocycline in MDR and XDR *P. aeruginosa* isolates[46].

(*iii*) Tobramycin-EPIs conjugate hybrid: The EPIs function by either competing with antibiotic binding site of efflux pump or by malfunction or interruption of OM channel to block the function of efflux pumps. Several EPIs such as 1-(1-naphthylmethyl)-piperazine (NMP), paroxetine (PAR), and dibasic peptide analogue of MC-04,124 (DBP) are covalently joined with tobramycin to form hybrid antibiotic. NMP has broad-spectrum action and potentiates tetracycline, fluoroquinolones, macrolides, penicillins and rifampicin in most Gram-negative bacteria except *P. aeruginosa*. PAR synergizes with tetracycline and fluoroquinolones in most Gram-negative and Gram-negative bacteria except *P. aeruginosa*. DBP potentiates fluoroquinolones in *P. aeruginosa*. The hybrid of tobramycin with EPIs augments the synergy and efficacy of EPIs and tetracycline combination against MDR Gram-negative bacteria including *P. aeruginosa*. In addition, development of resistance against tetracycline is suppressed, thereby providing an effective strategy to potentiate the legacy of tetracycline in MDR Gram-negative bacteria[47].

Cephalosporin hybridized with vancomycin

TD-1792 or cefilavancin is a heterodimer antibiotic consisting of vancomycin covalently linked through a stable linker to a third generation cephalosporin antibiotic molecule. The proposed modes of action are two distinct targets simultaneously, both of which participate in cell wall synthesis [D-Ala-D-Ala containing peptidoglycan precursors and the active site of penicillin-binding proteins (PBPs)][48]. TD-1792 is active in the presence of coexisting resistance mechanism including methicillin resistance in staphylococci, fluoroquinolone resistance in streptococci, penicillin resistance in pneumococci and quinupristin-dalfopristin resistance. It was more potent than cephalosporin and other glycopeptide antibiotics against MDR Gram-positive bacteria such as MSSA, MRSA, and heterogeneous VISA (hVISA). TD-1792 has superior efficacy than daptomycin, vancomycin and linezolid in the treatment of MRSA due to its prolonged post-antibiotic effect[48]. In phase II trial, to assess the safety and effiveness of TD-1792, in the treatment of complicated skin and soft-tissue infections, caused by Gram-positive organisms showed TD-1792 had comparable cure rates as that of vancomycin. The highest cure rate was seen in patients with MRSA infection. No serious adverse effects have been seen with TD-1792 and it is primarily excreted in urine. A Phase III trial was currently on going and the results are duly anticipated[49].

TD-1607 is another novel heterodimer antibiotic composed of glycopeptide covalently linked to 3rd generation cephalosporin. This hybrid has structural similarity to TD-1792. Its potent bactericidal activity is proposed to be through inhibition of cell wall biosynthesis[29]. In a study, TD-1607 showed good *in vitro* bactericidal properties against >1000 MRSA isolates (obtained worldwide, from patients with skin and soft tissue infections). Two phase I studies to assess safety, pharmacodynamics and pharmacokinetics parameters have been completed and study results are awaited[29].

Antibacterial hybrid with a non-antibacterial component

Antibiotics hybridized with efflux pump inhibitor (EPIs): A novel hybrid of antibiotic scaffolds with known EPI activity has been developed. It covalently links ciprofloxacin with a phenolic flavonoid, naringenin. Several flavonoids such as naringenin, quercetin, kaempferol, chrysin and genistein, are effective inhibitors of the multidrug transporters

from the MRP family[50]. The ciprofloxacin hybrid with naringenin inhibits DNA gyrase more (23-fold) than ciprofloxacin alone. This hybrid has significant activity against MRSA, MDR *E. coli* and amphotericin B-resistant *Candida albicans*[50]. In addition, the intracellular concentration in MRSA of this hybrid is five times higher than that of ciprofloxacin. Therefore, both high antibacterial action and intracellular concentration was attributed to inhibition of efflux pump. The antibacterial action was attributed by the ciprofloxacin pharmacophore and stronger DNA gyrase interaction was due to improved physiochemical property of naringenin[50].

Antibiotics hybridized with siderophores: The knowledge of covalently attaching a siderophore pharmacophore to an antibiotic pharmacophore uses the "Trojan horse" approach. This antibiotic hybrid misleads the bacteria to actively transport the antibiotic into the cell. Under low-iron concentration like in the human host, siderophores which are high-affinity iron chelators produced by bacteria are excreted. These siderophores scavenge ferric ions and the complex is taken up through active transport systems. By tagging an antibiotics to an iron chelating siderophore, intracellular drug concentrations are attained by hijacking the bacterial iron transport system[51,52]. The various points to be taken care of when designing siderophore linked antibiotics are use of non-native siderophore with low iron affinity, stable inker which remains stable in extracellular environment but releases the drug intracellular by enzymatic action and the siderophore uptake should not be hampered. Some of the antibiotic hybrids with siderophores are described below:

(*i*) Cefiderocol - This hybrid is made of the catechol 2-chloro-3, 4-dihydroxybenzoic acid linked covalently through a non-cleavable linker to cephalosporin ceftazidime. It shows strong and effective activity against Gram-negative ESKAPE pathogens. It showed potent action against carbapenem-resistant *P. aeruginosa* and *A. baumannii* and carbapenem-resistant *Enterobacteriaceae*[53]. In addition, cefiderocol possesses better potency and improved β-lactamase stability than many β-lactam and meropenem against organisms producing Groups A, B and D carbapenamases[53]. The superior antibacterial activity of ceftazidime is credited to the covalently attached catechol pharmacophore. It is being investigated for treatment of carbapenem-resistant Gram-negative bacterial infection and complicated urinary tract infections. Currently, cefiderocol is in phase 3 trials for its efficacy in the treatment of carbapenem-resistant Gram-negative pathogens and in comparison with meropenem for the treatment of nosocomial pneumonia[54].

(*ii*) In Enterobactin-ampicillin/amoxicillin antibiotic hybrid (Ent-Amp/Ent-Amx) β-lactam antibiotics are linked to enterobactin scaffold through a stable linker. Under conditions of iron limitation, enterobactin showed enhance activity against uropathogenic *E. coli* CFT073 and UT189, Enterohaemorrhagic *E.coli* 0157:H7 and Enterotoxigenic *E. coli* 078:H11. Ent-Amp/Ent-Amx showed 1000- fold decrease in MIC for uropathogenic *E. coli* CFT073 as compared to parent β-lactam and selectively killed *E. coli* CFT073 when co-cultured with *S. aureus*[55].

(*iii*) BAL 30072 is a recently developed hybrid antibiotics of siderophore with β-lactam conjugate. This monocyclic β-lactam contains an iron-chelating dihydroxypyridone substituent. The molecule has potent *in vitro* activity against many species of MDR isolates of the *Enterobacteriaceae* family including those producing Class A carbapenamases or a metallo-β-lactamase, MDR *P. aeruginosa, Acinetobacter* spp. and *Burkholderia pseudomallei*, including many carbapenem-resistant strains[56]. The synergistic effects of BAL30072 and carbapenems on antimicrobial activity have been observed, particularly in *Enterobacteriaceae* and *P. aeruginosa* due to the affinity of both molecules for the membrane-bound PBPs in the strains tested. Studies to investigate the clinical efficacy of BAL30072 in the treatment of complicated UTIs caused by multidrug-resistant *Enterobacteriaceae* are warranted[56].

Antibiotics hybridized with novel molecule

Aspergillomarasmine A (AMA) is a rapid and potent inhibitor of *metallo-β-lactamases* (MBL) such as NDM-1 and VIM-2[57]. The mode of inhibition of AMA is removal of active site metal ions essential for β-lactam hydrolysis. This is the first clinical inhibitor of MBLs that fully restores the activity of meropenem against NDM and VIM possessing Gram-negative bacteria such as *P. aeruginosa* and *Acinetobacter* species when treated with meropenem with AMA[58].

SPR741 is a cationic peptide derived from polymyxin which functions as an adjuvant to potentiate antibiotics action against Gram-negative pathogens. It exhibits minimal antibacterial activity but retains the ability to permeabilise the OM of Gram-

negative bacteria. In addition, it has reduced positive charge and lacks the lipophilic fatty acid side chain; therefore, there is no dose-dependent nephrotoxicity[59]. Various studies showed that SPR741 potentiated and extended the spectrum of activity of various antibiotics including fusidic acid, clarithromycin and rifampin in *Enterobacteriaceae* and *A. baumannii* but had no effect in *P. aeruginosa*[59]. In addition, o the recently concluded Phase 1 clinical study regarding the safety and tolerability of this hybrid antibiotics in healthy volunteers yielded favourable results[59].

Conclusion

MDR and XDR Gram-negative bacteria are a significant global health problem with no new drugs in treatment pipeline. This is due to multiple reasons, most important being the presence of an impermeable OM, efflux pumps and resistance mediated plasmids. In addition, due to overuse and misuse of antimicrobial agents, the resistance rates to current therapeutic agents have increased to alarming rates. The concept of antibiotic hybrids is remarkable in view of their benefits, but these are not without shortcomings. To create a concrete mode of action and benefits of antibiotics hybrid in clinical use, the molecular intricacy, challenging chemical synthesis, and the rigorous clinical trials need to be done. A few hybrids are currently in clinical trials. Cadazolid, a non-cleavable heterodimer consisting of ciprofloxacin and tedizolid, has completed phase 3 clinical trials in the treatment of *C. difficile*-associated diarrhoea *vis-a-vis* vancomycin. MCB-3681, an oxazolidinone and fluoroquinolone pharmacophores also for *C. difficile* infection, is in phase 2 clinical trial. CBR-2092 a rifamycin quinolone hybrid used for the treatment of acute bacterial skin, soft-tissue infections, and bacteraemia, is also in clinical trial. Cefilavancin (vancomycin and third generation hybrid, active against MRSA, hVISA) and cefiderocol (catechol 2-chloro-3, 4-dihydroxybenzoic acid and ceftazidime hybrid, active against carbapenamases producing Gram-negative bacteria) are also running in phase 3 clinical trials. In the coming years, more hybrid antibiotics will go into trials and eventually for patient treatment.

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