Trojan Horse Antibiotics—A Novel Way to Circumvent Gram-Negative Bacterial Resistance?

Glenn S. Tillotson

GSTMicro LLC, Durham, NC, USA.

ABSTRACT: Antibiotic resistance has been emerged as a major global health problem. In particular, gram-negative species pose a significant clinical challenge as bacteria develop or acquire more resistance mechanisms. Often, these bacteria possess multiple resistance mechanisms, thus nullifying most of the major classes of drugs. Novel approaches to this issue are urgently required. However, the challenges of developing new agents are immense. Introducing novel agents is fraught with hurdles, thus adapting known antibiotic classes by altering their chemical structure could be a way forward. A chemical addition to existing antibiotics known as a siderophore could be a solution to the gram-negative resistance issue. Siderophore molecules rely on the bacterial innate need for iron ions and thus can utilize a *Trojan Horse* approach to gain access to the bacterial cell. The current approaches to using this potential method are reviewed.

KEYWORDS: siderophore, antibiotic resistance, gram negatives, klebsiella pneumoniae carbapenemase (KPC), MBL

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CORRESPONDENCE: gtillotson@cempra.com; gtillotsonconsult@yahoo.com

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Introduction

Marc Sprenger, Director of the WHO's secretariat for antimicrobial resistance, recently stated that "Many such infections are rapidly becoming resistant to life-saving drugs";[1] thus, we may be on the verge of the post-antibiotic era. Indeed, it was also proposed that many of the procedures and conditions such as simple operations or cancer immunosuppression, which we take for granted today, may become impossible due to these organisms.[2]

Extended-spectrum beta-lactamases (ESBL) have become a global scourge in the past 20 years. Initially thought to be only nosocomial problems have now become commonplace in community-acquired infections. It has been predicted that soon ESBL-producing *Escherichia coli* will be as common as methicillin-resistant *Staphylococcus aureus* (MRSA). The dissemination of ESBL mechanisms has been facilitated by the spread of plasmids, which may in fact carry other multiple resistance mechanisms. Recent surveillance programs have illustrated the frightening scale of the presence of ESBLs, from 55%–65% in China to 67%–79% in India. Perhaps most worrying is the report that 96% of *Klebsiella pneumoniae* were ESBL producers with 50% from community infections.[3] Although rates of these pathogens are lower in the US and most of Europe, the rapid expansion of global travel leads us to realize that an infection may initiate anywhere and be manifested elsewhere.

There are three definitions of resistance, which apply to more and more pathogens; these are multidrug resistant (ie, resistant to at least three different drug classes), extensively drug resistant (ie, resistant to all but one or two drug classes), and pan resistant (ie, resistant to all approved antibiotics).[4] All of these infections are very challenging from a clinical perspective, besides the escalating epidemiological issues. More recently, the spread of carbapenem-resistant enterobactericeae (CRE) has become a global issue. The carbapenem class of antibiotics has become the *go-to* group of drugs in light of ESBLs, which are increasing in frequency and diversity. However, in the face of carbapenem resistance, only colistin has, until recently, been a reliable last resort treatment. Moreover, a major recent development has been the emergence of colistin-resistant strains from China; this mechanism is potentially a global threat and as such is the first sign of our last therapeutic weapon becoming less effective.[5]

In particular, it is the recent escalation of multiple mechanisms of resistance among gram-negative species, which are causing the greatest concern. These species go beyond *Pseudomonas aeruginosa* or *Acinetobacter baumannii*, which are acknowledged to be major problems, but now include various members of the Enterobacteriaceae notably *E. coli*, *K. pneumoniae*, and *Enterobacter cloacae*. These species often harbor multiple mechanisms of resistance to classes of antibiotics as diverse as fluoroquinolones, aminoglycosides, tetracyclines, and β-lactams. Indeed, the latter class can be rendered ineffective due to multiple methods of resistance including enzymes that can destroy many β-lactams, altered cell wall porins, and

increased efflux mechanisms, which adversely regulate the entry or exit of antibiotics.[6] It has been the recognition of the emergence of the β-lactamase enzymes, which has caused huge concerns. In response to exposure to extensive use of various penicillins and cephalosporins, bacteria have evolved and disseminated over 1,300 different hydrolyzing enzymes, most of which can be transferred from one bacterial species to another by virtue of plasmids or similar genetic methods.[7] These multidrug-resistant species can then spread globally by virtue of rapid air travel across continents. A good example is the recent NDM-1-containing *Klebsiella* strains from India to Europe and beyond. Although several β-lactam–β-lactamase combinations have been or are in development such as ceftazidime/avibactam and ceftolozane–tazobactam, they do not cover all the various classes of β-lactamase enzymes. There are gaps in spectra of many of the current compounds (Table 1). Thus, it has been proposed that one way to avoid or, at least, reduce the damage of β-lactamase enzymes outside the bacterial cell would be to ensure that the drugs are rapidly able to access the intracellular spaces and withstand internal β-lactamase enzymes.[8] Moreover, in addition to neutralizing the destructive effects of diverse β-lactamase enzymes, the outer membrane of the gram-negative cell wall poses a significant hurdle. Various species can downregulate certain outer membrane proteins to exclude certain antibiotics, including β-lactams. Examples of these altered outer membrane proteins include OprD, OmpK, and CarO.[9] In addition to prevention of cell access, efflux pumps are another significant mechanism of bacterial resistance. Bacterial efflux pumps are divided into five groups, namely, the major facilitator superfamily, the small multidrug-resistant (MDR) family, the multidrug and toxic compound extrusion family, the ATP-binding cassette family, and the resistance-nodulation-cell division family. The latter is the most clinically relevant in terms of antibiotic resistance.[10]

It has been hypothesized that it may be possible to harness a vital bacterial survival mechanism to enable access to the bacterial cell. Such a process depends on the need for the essential element, iron. Bacteria secrete aggressive iron-complexing proteins known as siderophores, which scavenge iron from their environment to survive. This process could be compared to mining by solubilizing iron ions in mineral form or biologically complexed iron ions such as iron bound to transferrins. Siderophores (from the Greek *iron carriers*) can strip iron ions out of these situations and create a complex of iron and protein; this iron-siderophore is recognized by specific bacterial uptake systems, which enable the binding of the complex to outer membrane receptors. These complexes are taken up and then released into the periplasmic space and into the cytoplasm. There are several siderophore agents that operate in the same manner, but use slightly different carrier molecules, eg, microcins, sideromycins, and natural siderophores such as ferrimycin.

Human interest in this *smuggling* approach to conveying iron and other molecules into cells began over 50 years ago. Indeed, this approach has been exploited in several therapeutic regimens including cancer therapies. This method of conveying compounds into other cells such as bacteria has been likened to the *Trojan Horse* legend. Greek mythology tells the story of the war between the Greeks and Trojans. Legend has it that Odysseus built a huge wooden horse, the emblem of the Trojans; this enabled Greek soldiers to be carried within the wooden horse and into the city of Troy, allowing the Greeks to attack Troy from within. This technique became known as the Trojan Horse.[11] It is this analogy that is used by current antibiotic developers in an effort to overcome gram-negative resistance. A major hurdle in creating effective gram-negative antibiotics is the need to cross the outer membrane into the gram-negative periplasmic space. Figure 1 illustrates the concept of coupling of iron ions to the siderophore–cephalosporin complex and then transport of the complex into the periplasmic space in which the released cephalosporin attaches to the critical cell wall penicillin-binding proteins. Beyond the periplasmic space, the siderophore complex enables β-lactams to penetrate into the cell via passive transportation. β-lactam antibiotics have been the most studied class as a possible carriers for siderophore conjugates, but the concept can also be applied to other classes of antibiotic including fluoroquinolones where cellular access has been reduced or stopped due to porins or efflux mechanisms. β-lactam core molecules include monobactams, cephalosporins, and monocarbams. The side chains added to the central active agent can be quite varied with catechols, which seem to be the most effective.

However, there are significant technical issues with susceptibility testing of these agents in that the standardized

Table 1. Overview of current-/late-stage developmental gram-negative agents.

DRUG	STAGE	β-LACTAMASE ACTIVITY	GAPS	REFERENCES
Ceftazidime/avibactam (AVYCAZ)	Approved	ESBL; TEM, SHV; KPC; ampC, some OXA, some ampC in *P. aeruginosa*	MBL and efflux or porins	36
Ceftalozane/tazobactam (ZERBAXA)	Approved	ESBL; TEM, CTX-M, OXA; *P. aeruginosa*-ampC, OprD, MexXY	KPC, MBL	37
Meropenem/RPX7009	Phase 2	KPC, ampC (most), ESBL; TEM, SHV	KPC ompK 35 or 36	38
S649266	Phase 2	KPC, NDM-1, ESBL VIM, IMP, CRE, MBL	None reported	18,19

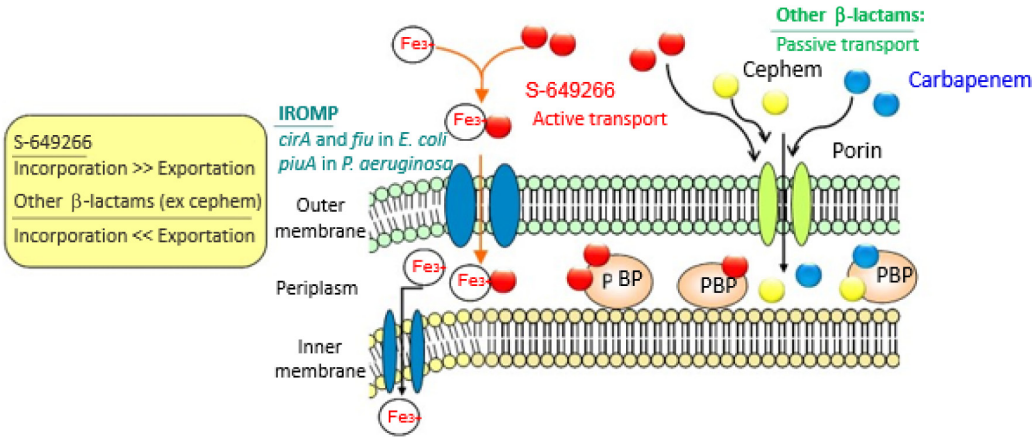

Figure 1. Depiction of siderophore action.
Abbreviation: PBP, penicillin-binding protein.

methods, eg, Committee for Laboratory Standards Institute or European Committee for Antimicrobial Susceptibility Testing, as they require specific media, such as Mueller Hinton Broth (MHB) supplemented with certain ions such as calcium and magnesium. However this medium, and possibly other test media, contains levels of iron, which are not representative of physiological concentrations of ferrous or ferric ions. Thus, in standard media, minimum inhibitory concentrations (MICs) tend to be 4–32 times higher than in iron-depleted settings. There have been two approaches to removing these iron ions by using cation-binding resins, which are added to MHB. The two tested agents are apo-T (solid media)[12] and Chelex (broth media).[13] These agents remove all ionic components but require subsequent addition of essential zinc, calcium, and magnesium ions to be supplemented to the test media after cation-binding treatment. These laboratory methods have been validated by in vivo or animal infection models, which are clearly depleted in terms of free iron ions.[14] This technical approach will need to be overcome once the drugs move into late clinical development as routine approaches will yield inaccurate and inappropriate, misleading high MICs.

Currently, there are three such *Trojan Horse* complexes in development. Each has a different structure, but all are based on siderophore technology, thereby gaining access to the bacterial cell. These agents are MC-1, a siderophore-conjugated monocarbam, from Pfizer; BAL30072, a siderophore monosulfactam, from Basilea; and S-649266, a catechol–cephalosporin antibiotic, from Shionogi Pharmaceuticals. In vitro activity is the initial measure of the potential of a compound, and for the three examples of siderophore agents, in vitro data are presented in Tables 2 and 3. The reported studies focus on both routine clinical isolates and specific genetically modified species such as *P. aeruginosa*. To date, there have been no *hea1-head* in vivo comparisons reported as all three agents are in clinical development, although they are at different phases and are not readily available.

MC-1

MC-1 is a novel siderophore-conjugated monocarbam antibiotic, which has shown activity against MDR *P. aeruginosa* and ESBL-producing members of the Enterobacteriaceae. McPherson et al[15] examined the in vitro activity of MC-1 against an isogenic library of strains of *E. coli*, which were created by synthesizing a representative β-lactamase from each class and then using that clone as a template for further mutagenesis to construct the desired genetic variants. Table 2 shows the activity of MC-1 alone and in combination with the commonly used β-lactamase inhibitor tazobactam and against BAL30072 (a siderophore monosulfactam), aztreonam, ceftazidime, cefepime, and meropenem against a selection of clinically relevant β-lactamase enzymes. MC-1 showed MIC_{90} values of 0.06–0.25 mg/L including metallo β-lactamase (MBL) strains that exhibited high MICs to meropenem, cefepime, and ceftazidime.

Additionally, an isogenic panel of *P. aeruginosa* was constructed to estimate the cellular entry of MC-1; a total of 30 mutants were examined against MC-1, BAL30072, and

Table 2. In vitro activity (MIC_{90}) of MC-1 and BAL 30072 compared with other β-lactam agents.[15]

β-LACTAMASE	MC-1	BAL30072	AZT	MER	CPM	CTZ
SHV	0.25	>64	>64	0.03	1.0	64
TEM	0.06	16	32	0.03	1.0	32
KPC	0.06	1.0	32	0.06	0.25	8
CTX-M	0.25	1.0	>64	0.25	8	32
GES	0.06	1.0	0.5	0.25	0.5	>64
VEβ	0.5	16	32	0.25	0.5	>64
MβL	0.125	0.25	0.5	16	4	>64
OXA	0.25	0.25	1.0	0.03	0.125	0.5

Abbreviations: AZT, aztreonam; MER, meropenem; CPM, cefipime; CTZ, ceftazdime.

Table 3. In vitro activity of S-649266.

ORGANISM (NO. OF ISOLATES)	MIC_{90} (μg/mL)			
	S-649266	CEFEPIME	PIPERACILLIN/TAZOBACTAM	MEROPENEM
ESBL producers				
E. coli (50)	0.25	>64	128	0.063
K. pneumoniae (50)	0.5	>64	>256	0.125
E. cloacae (10)	4	>64	128	0.5
MBL producing *P. aeruginosa* (33)	4	>64	256	>32
Multidrug resistant				
P. aeruginosa (30)	1	>64	>256	>32
A. baumannii (30)	4	>64	>256	>32
NDM-1 producers (50)	4	>32	–	>16
KPC producers (47)	0.5	>64	>256	>32

aztreonam. Consistently, MC-1 was the most active agent tested with MICs in iron-low medium in the order of 0.25–1 mg/L, while BAL30072 showed MICs of >2–64 mg/L and aztreonam 4 or >4 mg/L.[15] The authors concluded that MC-1 was active against porin-mutated strains of *P. aeruginosa* and a wide range of gram-negative-resistant strains including those having β-lactamase and porin alterations. An interesting observation that is common to siderophore studies was the discordance between in vivo murine septicemia model and in vitro MICs in standard susceptibility test media MHB. However, the in vivo response did correlate with iron-depleted MHB. This shift has been accommodated in pharmacodynamic studies and early clinical studies. This drug does not appear to be in clinical development according to ClinicalTrials.gov (August, 2016), and thus, no human tolerability data are available.

BAL30072

BAL30072 is a monosulfactam conjugated with an iron-chelating dihydroxypyridone moiety, which was developed by Basilea Pharmaceutica. It has been investigated in combination with a range of antibiotics commonly used against gram-negative organisms, but with the emergence of multidrug-resistant strains, they are less active. Gram-negative pathogens with β-lactam-resistant phenotypes were evaluated and compared with the activities of reference drugs, including aztreonam, ceftazidime, cefepime, meropenem, imipenem, and piperacillin/tazobactam.

BAL30072 showed potent activity against MDR *P. aeruginosa* and *Acinetobacter* sp. isolates, including many carbapenem-resistant strains. The MIC_{90}s were 4 μg/mL for MDR *Acinetobacter* spp. and 8 μg/mL for MDR *P. aeruginosa*, whereas the MIC_{90} of meropenem for the same sets of isolates was >32 μg/mL.[16] Table 2 shows these results.

BAL30072 was bactericidal against both *Acinetobacter* spp. and *P. aeruginosa*, even against strains that produced metallo-β-lactamases that conferred resistance to all other β-lactams tested, including aztreonam. The compound was also shown to trigger spheroplast formation and lysis as opposed to extensive filamentous formation. This is probably due to the points of interaction with three penicillin-binding proteins such as PBP1a, PBP1b, and PBP3. BAL30072 was subsequently tested in combination with imipenem, meropenem, and doripenem against selected strains of Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii* using 1 mg/L of each combination. Broadly these showed activity against 70%–80% of strains, whereas the carbapenems alone were ineffective and BAL30072 was only 20%–40% effective. Synergistic effects were seen in most species except *A. baumannii*. No antibiotic combinations were antagonistic. A murine model of septicemia supported the enhanced synergy of meropenem and BAL30072.[17]

In the direct comparison of BAL30072 with MC-1, the latter agent was more active in vitro against all enzyme isogenic strains, although the difference among metallo-β-lactamases and OXA strains was similar having MIC_{90}s of 0.125 and 0.25 mg/L, respectively.[15] This drug was not listed in current clinical trials (ClinicalTrials.gov accessed August 2016); thus, no human tolerability data are available.

S-649266 (Cefiderocol)

S-649266, or cefiderocol, is a novel siderophore cephalosporin antibiotic with a catechol moiety on the 3-position side chain (Fig. 2). Two sets of recent clinical isolates were used to evaluate the antimicrobial activity of S-649266 against

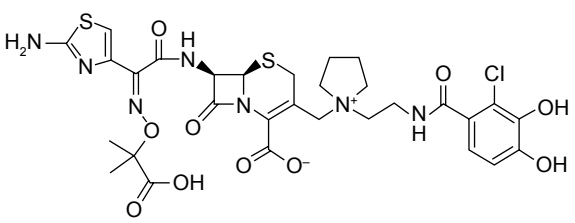

Figure 2. S-649266 (cefiderocol).

Enterobacteriaceae. These sets included 617 global isolates collected between 2009 and 2011 and 233 β-lactamase-identified isolates, including 47 KPC, 50 NDM, 12 VIM, and 8 IMP producers.[18] The MIC_{90} values of S-649266 against the first set of *E. coli*, *K. pneumoniae*, *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter aerogenes*, and *E. cloacae* isolates were all ≤1 μg/mL, and there were only 8 isolates (1.3%) among these 617 clinical isolates with MIC values of ≥8 μg/mL, see Table 3. S-649266 was evaluated against gram-negative bacteria, including MDR strains and carbapenem nonsusceptible strains, and compared with cefepime, piperacillin/tazobactam, and meropenem. MIC_{90} values of S-649266 were 1–4 μg/mL against MDR *P. aeruginosa*, MDR *A. baumannii*, metallo beta-lactamase-producing *P. aeruginosa*, and NDM-1 producers.[18,19] On the other hand, MIC_{90} values of comparators were >16 μg/mL. MIC_{90} values of S-649266 against carbapenemase nonproducing ESBL producers of *E. coli*, *K. pneumoniae*, and *E. cloacae* were 0.25, 0.5, and 4 μg/mL, respectively. While the MIC_{90} values of cefepime and piperacillin/tazobactam were ≥32 μg/mL.

The antibacterial activity of S-649266 against carbapenemase producers and its stability against clinically relevant carbapenemases were also investigated. The catalytic efficiencies (k_{cat}/K_m) of IMP-1, VIM-2, and L1 for S-649266 were 0.0048, 0.0050, and 0.024 μM^{-1} s^{-1}, respectively, which were more than 260-fold lower than that for meropenem. Only slight hydrolysis of S-649266 against KPC-3 was observed. NDM-1 hydrolyzed meropenem threefold faster than S-649266 at 200 μM.[20]

It is increasingly appreciated that adaptation is a major mechanism associated with the acquisition and evolution of antibiotic resistance. Adaptive resistance is a specific type of nonmutational resistance that is characterized by its transient nature. It occurs in response to certain environmental conditions or due to epigenetic phenomena like persistence. It has been proposed that this type of resistance could be the key to understanding the failure of some antibiotic therapy programs. However, adaptive resistance mechanisms are still somewhat unclear. Equally the genetics behind some of the changes involved in adaptive resistance may explain the phenomenon of *baseline creep*, whereby the average (MIC) of a given species increases steadily but inexorably over time, making the likelihood of breakthrough resistance greater.

Previous siderophore-based β-lactam compounds, such as monobactam (MB-1) and the monocarbam (SMC-3176), demonstrated inconsistent activity probably due to development of adaptive resistance.[21,22] Thus, Ghazi et al[23] examined S-64926, MB-1, and SMC-3167 in the neutropenic mouse model to determine the relative penetration through outer membrane via iron transporter systems as well as the stability of the three molecules against serine- and metallocarbapenemases. Using this established thigh infection model, *P. aeruginosa* was examined to explore the pharmacodynamic profile of these compounds with respect to efficacy and development of adaptive resistance. Eight clinical *P. aeruginosa* isolates were tested. MICs were determined by broth microdilution in triplicate (iron deficient) and modal MIC was reported. Groups of three mice were inoculated, and two hours later, they were treated with ascending doses of S-649266 or humanized doses of siderophore β-lactams MB-1 and SMC-3167, as determined in previous studies.[21,22] After 24 hours, the animals were sacrificed for bacterial enumeration and determination of the change in bacterial density (log10 CFU) relative to the starting inoculum. Unlike the previously reported variable efficacy with siderophores MB-1 and SMC-3175 against the *P. aeruginosa* studied, S-649266 displayed sustained antibacterial effects for all isolates over the treatment period. Enhanced bacterial kill was observed over the dose range studied, and as previously observed, $\%fT >$ MIC correlated well to the efficacy of S-649266. Ghazi et al proposed that catechol substitution may impart improved activity compared to other siderophore-conjugated β-lactams, suggesting that adaptive resistance was not observed in this model. Clearly broader clinical exposure will test these results, but based on these lower adaptive resistance results, S-649266 appears to have a lower potential for resistance selection.

A pharmacokinetic model providing probability of target attainment (PTA) data described the time courses of S-649266 concentrations in plasma and urine, which were used to predict efficacy for optimizing dosage regimens.[24] The simulations for the subjects with normal renal function suggested that S-649266 at 2 g q8h would exhibit efficacy for the target pathogens (ie, carbapenem-resistant *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*; MIC_{90}: 0.5, 2, 2, and 8 μg/mL, respectively). The 3-hour infusion would provide an adequate PTA, while patients on 1 g q8h dosing would probably be insufficient. This hypothesis was examined in a rat lung infection model.[24] The model described plasma and urine concentration data, which yielded PTA values with 2 g q8h with 1-hour or 3-hour infusion. For 2 g q8h with either infusion time, the PTA was >90% at 8 and 4 μg/mL of MIC for 50% and 75% of $fT >$ MIC, respectively. The PTA for both $fT >$ MIC targets at 2 g q8h with 3-hour infusion was higher than that observed with 1-hour infusion. The predicted fifth percentiles of urine concentrations over 8 hours were >100 μg/mL with 2 g with 1-hour infusion. The simulations for the subjects with normal renal function confirmed that a 2 g dose every 8 hours S-649266 would provide adequate efficacy for most target pathogens while a 1-hour infusion was likely to be inadequate, especially if the MICs were slightly elevated. Thus, 2 g given every 8 hours would probably achieve urinary concentrations likely to eradicate most MDR gram-negative pathogens.[24]

The clinical development of these three siderophore agents is less than clear. Only Shionogi Pharmaceuticals with S-649266 is registered on ClinicalTrials.gov with their Phase 2/3 program, which is comparison of S-649266 with imipenem/cilastatin for the treatment of complicated urinary tract infections in adults. This is a multicenter, double-blind

international study with a focus on enrolling patients with multidrug-resistant, carbapenem-susceptible, gram-negative pathogens.[25] There is no clinical trial information on either MC-1 or BAL30072. A novel (Phase 3) study of S-649266 will enroll only patients with evidence of carbapenem-resistant pathogens, regardless of the primary infection site. This *pathogen-focused* study will rely on rapid diagnostic technologies to identify eligible patients.

Conclusions

The onslaught of multidrug-resistant bacteria is a global problem with the emergence and geographical expansion as a major clinical threat. In the past few years, we have seen several novel approaches to combatting bacterial resistance; these include synthetic peptides, cationic antimicrobial peptides (CAMPs), lantibiotics, lipophosphoxins, nosokomycins, and β-sitosterol.

The synthetic peptides have been studied as potential antibacterial agents for over 20 years, a recent focus on multidrug-resistant gram-negative species. McGrath et al have engineered a synthetic peptide that shreds and dissolves the double-layered membrane, which are considered to be a prime defensive mechanism of gram-negative species. This spiral peptide called KLAKLAKKLAKLAK acts by puncturing this unique bacterial bilayer without affecting eukaryotic cells. However, these peptides are subject to normal host enzyme destruction of some enzymes excreted by the bacterium itself. Thus, in order to combat this negative impact, increase in dosing will be needed, which may bring increased toxicity and manufacturing costs. The authors showed in vitro activity against key nosocomial pathogens with a dose-dependent killing. This synthetic peptide eliminates biofilms that may be important in settings where bacteria establish microcolonies and then seed to cause infection such as bone and joint infections. Animal models are now required to fulfill the next steps of drug development.[26]

CAMPs are essential natural innate immune defense mechanisms that inhibit colonization by pathogens and aid in the clearance of infections. Gram-negative species are a major target but some evolved resistance mechanisms. Such mechanisms undoubtedly contribute to virulence and survival of pathogens. Over 1,200 natural CAMP molecules have been identified from prokaryotes and vertebrates. They possess certain constant features including cationic, amphipathic, and relatively hydrophobic. CAMPs destabilize the bilayer membrane by interacting with anionic head groups and hydrophobic fatty acid chains. This enables the CAMP to destabilize the membrane, leading to cell lysis. It has been hypothesized that CAMPs also have intracellular targets that also contribute to cell wall disruption and cell death. Two CAMPs are in clinical use, namely, polymyxin B and colistin (polymyxin E); however, bacterial resistance has recently been reported in China[27] and very recently in USA.[28] Bacterial resistance occurs via surface remodeling, usually lipopolysaccharide modification, capsule production, biofilms, efflux pumps, and proteolytic degradation.[29] Clearly with such an array of CAMP resistance mechanisms, this may limit the value of the class in the clinical setting. Moreover, the known renal toxicity of colistin is another hurdle to be overcome. It has been proposed that establishment of some of these CAMP resistance mechanisms are additions to the bacterial pathogenicity. Deeper understanding of the CAMP resistance mechanisms may help yield further gram-negative antibiotics.

In an effort to overcome some of these issues, Torcato et al[30] designed and characterized two new molecules, namely, R-BP100 and RW-BP100. These analogs have two amino acids, in which Tyr is replaced with a Trp and/or the Lys residues replaced with Arg. These analogs are active against a wide range of gram-negative species as well as, unusually for peptides, all tested gram-positive bacteria. Various studies show the target site of the bacterial membrane. These minor, but significant, structural changes may yield potential new class of antibacterial agents for a broad range of multidrug-resistant species.

Draper et al[31] examined posttranslationally modified ribosomally synthesized antimicrobial peptides, lantibiotics, with a broad-spectrum antimicrobial activity. One lantibiotic 3147 showed a wide range of anti-gram-positive activity. Draper et al tested the enhancement of 3147 with polymyxin B and E using synergism tests. Using low levels of a polymyxin, the lantibiotic 3147 activity against gram-positive and some gram-negative species. They hypothesized that use of 3147 may allow for use of lower, thus less, toxic concentrations of polymyxin.

Panova et al[32] discovered a new series of compounds termed lipophosphoxins (LPPOs), which showed specific activity toward gram-positive species. LPPOs are bactericidal in activity and localize to the plasma membrane in bacteria but not in eukaryotic cells. LPPOs create pores in the bacterial membrane. Of key concern with any new class of compound toxicity to humans is essential thus showing no genotoxicity in the Ames test, do not cross monolayer of Caco-2 cells and well tolerated by mice when given orally but not via the peritoneum. As the agents withstand low pH, it has been proposed that they may not be viable systemic antibiotics but may have a role as nonabsorbed antibiotics such as in *Clostridium difficile* of *Helicobacter pylori* agents.

MRSA is still a major nosocomial pathogen, although recent additions to the armamentarium such as modified glycopeptides and oxazolidinones have recently been approved for clinical use; it is undisputed that MRSA will find a way to resist these new agents. Tomoda[33] reported on a new member of the phosphoglycolipid family, the nosokomycins from *Streptomyces cyslabdanicus* using a silkworm model. The proposed target site is penicillin-binding proteins specific to MRSA. Although these data are very preliminary, they suggest a novel approach to inhibiting gram-positive cell wall production.

Li et al[34] reported the use of a phytosterol, β-sitosterol, to protect against cell lysis by pneumococcal pneumolysin, a potent virulence mechanism of *Streptococcus pneumoniae*. Mouse model studies showed the protection of cells from cholesterol-dependent toxins that contribute to pneumococcal infections.

There is clearly a huge amount of research into alternative methods to combat antibiotic-resistant bacterial infections. However, many of these molecules are still in their early stages with very few having been exposed to humans. Indeed, a few seem to have been tested in animal models. So although there is much excitement at these innovations, we have to turn to classes we understand and have a long-standing safety record.

Thus, we resort to the exploration of one of the oldest antibiotic classes, the β-lactams. Most recently, extension of the β-lactam/β-lactamase inhibitor combinations (eg, ceftazidime/avibactam or ceftolazane/tazobactam) and modified carbapenems are clinical options. Of particular interest is the recent announcement of the initial Phase 3 clinical study of meropenem–vaborbactam in complicated urinary tract infections, which was compared with piperacillin/tazobactam. Notably the clinical efficacy was 98.4%; yet, the microbiological eradication was 66.7% compared with 57.7% reported with piperacillin/tazobactam.[35] The second Phase 3 study comparing this new combination with *best available therapy* may be more instructive with regard to the strains producing inhibitor-resistant TEMs, complex mutant TEMs, or AmpC β-lactamases, which were found to be generally resistant to older inhibitor combinations, and the presence of these enzymes is probably due to the increased use of β-lactam/β-lactamase inhibitor combinations, which is escalating. Importantly, novel β-lactamase inhibitors do not address the multifactorial resistance mechanisms in gram-negative bacteria, particularly, *P. aeruginosa and A. baumannii*, which are mediated by porin mutations and efflux overproduction. Thus, with these changes, clinicians are turning to relatively toxic agents such as colistin or multiple drug combinations to manage infections such as pneumonia, bacteremia, wound, urinary tract, and other serious systemic infections. The utility of siderophore antibiotics by virtue of their activity against an array of ESBLs and cell access mechanisms is an encouraging development and may be one that clinicians are in need of, but we await the initial clinical findings with S-649266.

Author Contributions

Conceived and designed the experiments: GT. Analyzed the data: GT. Wrote the first draft of the manuscript: GT. Contributed to the writing of the manuscript: GT. Agree with manuscript results and conclusions: GT. Jointly developed the structure and arguments for the paper: GT. Made critical revisions and approved final version: GT. Author reviewed and approved of the final manuscript.

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