Daptomycin: Discovery, Development and Perspectives

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The emergence of antimicrobial drugs is a breakthrough health intervention that has helped save millions of lives and reduces the morbidity of several infectious diseases. However, the concrete advances in control of infectious diseases need to tackle the parallel upsurge in resistance to antimicrobials. There has been an alarming situation in the healthcare industry today, since antibiotic resistance has become a serious clinical issue. The armory of antibiotics is becoming less efficient leaving only few dependable replacements. The healthcare sector is intimidated by the labor and costs of putting an entirely new molecule in the market. The reliability of developing new anti-infective molecules is being questioned. Methicillin-resistant *Staphylococcus aureus* (MRSA) has challenged many potential antibiotics. MRSA may lead to deactivation and inefficacy of antibiotics. The increased use of vancomycin and linezolid, effectual antibiotics against Methicillin-resistant *Staphylococcus aureus* (MRSA) has resulted in resistant isolates. Amongst few novel antibiotics, lipopeptide antibiotics have emerged as stars of the recent times. Daptomycin is a promising member of the lipopeptide antibiotic family, which has displayed a broad spectrum of activity in vitro against a wide range of gram-positive bacteria, including Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci. Daptomycin is not affected by mechanisms that confer specific resistance to beta-lactam agents (including methicillin), glycopeptides (such as vancomycin), quinupristin/dalfopristin, linezolid or other agents potentially useful against Gram-positive bacteria species. The unique mechanism of action and low resistance profile, together with rapid bactericidal action make Daptomycin a promising alternative in future to act against resistant organisms.

Keywords: Daptomycin; multi-drug resistance; methicillin-resistant *Staphylococcus aureus* (MRSA); cyclic lipopeptide antibiotic

1. Introduction to Daptomycin

Antibiotic resistance has become a serious clinical issue in today's world. The failure of generations of antibiotics to combat "Superbugs" has threatened the healthcare sector. Though several antimicrobials are being worked upon but still there is an uncertainty regarding their efficacy and cost-effectiveness [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has challenged many established antibiotics [2]. The increased use of popular antibiotic against Methicillin-resistant *Staphylococcus aureus* (MRSA) like Vancomycin has resulted in resistant isolates. The smart antibiotic resistant strains have prevented vancomycin from reaching the target nascent cell wall precursors through thickening of their cell walls [3]. There is a need for potential antibiotics against MRSA activity with a modified mechanism of action [4]. Lipopeptide antibiotics are novel antibiotics against MRSA with unique mechanism of action [5]. They are produced by soil actinomycetes *via* non-ribosomal biosynthetic pathways. Their structure consists of an acyl chain conjugated to a linear or cyclic peptide sequence; the peptide portion can either have cationic or anionic residues [6]. Daptomycin is a promising member of the lipopeptide antibiotic family, which has displayed a broad spectrum of activity in vitro against a wide range of gram-positive bacteria, including Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci. The mechanism of its action involves calcium-dependent dissipation of membrane potential leading to the release of intracellular ions from the cell and bacterial death [7].

1.1 The Challenge of Methicillin Resistant Microorganisms

Methicillin resistant microorganisms have posed threat to the life-saving drugs from a long time. The advent of the first penicillin-resistant cocci in 1944 came as a serious challenge [8]. Methicillin came into existence in 1956 and only two years later the first Methicillin-resistant strain of *Staphylococcus aureus* (MRSA) had been isolated [9]. Since then, there has been a tremendous increase in the number of antibiotic resistant strains. Studies suggest the prevalence of resistant isolates in the US where 59% from skin and soft tissue infections were resistant to Methicillin [10]. In France , 3.6%, while in Greece- 75% of MRSA strains were reported [11, 12] .Vancomycin, a tricyclic glycopeptides produced by *Streptomyces orientalis* was discovered in 1956. It has been a popular drug for a long time in the treatment of MRSA infections [13]. Vancomycin has side-effects, like nephrotoxicity and ototoxicity [14]. In 1996 the first vancomycin-resistant *S. aureus* (VRSA) was detected. The thickening in the bacterial cell wall has hampered the efficacy of vancomycin [15]. In order to combat such problems, proper drug monitoring is required [16]. Linezolid is another valuable drug which is the first marketed antibiotic of the oxazolidinone class demonstrated activity against antibiotic-susceptible and antibiotic-resistant aerobic Gram-positive cocci. Though, Linezolid can be orally administered but it has few known side effects, e.g., bone marrow depression and poses higher risks to patients in case of solid organ

transplantation. In this context, there is a need for a drug that is effective on MRSA and has minimal side-effects [17]. Owing to these conditions, Daptomycin has received much attention as a potential anti-infective drug.

Table 1 A comparative study of Daptomycin with other popular anti-MRSA drugs [18].

Properties	Vancomycin	Daptomycin	Linezolid
Class of antibiotics	Glycopeptide	Lipopeptide	oxazolidinone
Mode of Action	Inhibits cell-wall synthesis	Calcium-dependent dissipation of membrane potential	Inhibits protein synthesis
Applications	Severe infections caused by susceptible strains of methicillin-resistant *S.aureus*. As a combinatorial drug with aminoglycoside for endocarditis.	Complicated skin and skin structure infections. S.aureus bloodstream infections (bacteremia),including those with right-sided infective endocarditis, caused by MRSA isolates	Complicated skin and skin structure infections including diabetic foot ulcers caused by MSSA. Treats Vancomycin Resistant *E.faecium* infections
Bioavailibility	Incomplete absorption	Complete absorption	Incomplete absorption
Clearance	0.06L/h/kg	0.10L/h/kg	0.01L/h/kg
Volume of Distribution	0.3 to 0.43 L/kg	0.7-0.8 L/kg	0.1L/kg
Half Life	4-6h	4-5h	8h
Protein Binding	55%	31%	93%
Tissue penetaration and effects	Average	High	Low
Dosing Therapy	1000mg IV over 60 min. Q12h with monitoring	600mg IV over 30-120 min. Q12H 400-600 mg PO Q12h	Increased dose interval

1.2 Discovery of Daptomycin

Daptomycin is the first approved member of the A21978C family of the cyclic anionic 13-amino acid lipopeptide antibiotics, produced as a secondary metabolite by *Streptomyces roseosporus*. It was initially developed in the late 1980s and early 1990s, at Eli Lilly and Company, by supplying decanoic acid to the growth media of Streptomyces roseosporus during fermentation, but was held over due to concerns regarding myopathy. Cubist Pharmaceuticals Inc. licensed worldwide rights from Eli Lilly and Company in 1997[5]. Daptomycin is a cyclic lipopeptide which consists of a 13-member amino acid cyclic lipopeptide (hydrophilic core) with a decanoyl side chain (lipophilic tail) as shown in Figure 1. The empirical formula of Daptomycin is C72H101N17O26; the molecular weight is 1620.67, while its empirical name is N-decanoyl-L tryptophyl-D-asparaginyl-L-aspartyl-L-threonylglycyl-L-ornithyl-L-aspartyl-D-alanyl-L aspartylglycyl-D-seryl-threo-3-methyl-L-glutamyl-3-anthraniloyl-L-alanine ε1-lactone[18].The initial trials dealt with a dose of 4 mg twice daily and had to be stopped in 1991 because of frequent elevations of serum levels of creatine kinase (CK) probably related to skeletal muscle toxicity[18]. In 1999 the drug was re-introduced into clinical trials. The FDA approved Daptomycin for the treatment of cSSSIs at a dose of 4 mg/kg daily (preceded by a starting dose of 6 mg/kg/ day) at last in 2003. In 2006 an additional FDA approval was granted for the treatment of bloodstream infections and right-sided endocarditis caused by methicillin-sensitive *S. aureus* (MSSA) and MRSA. The approval was confirmed in Europe in 2007[19].

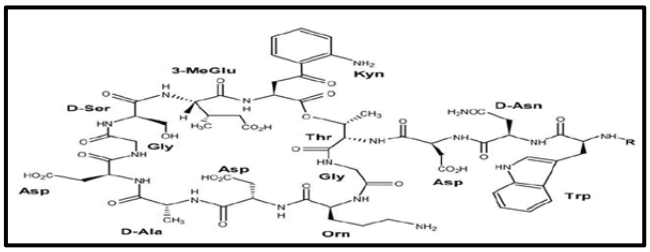

Fig. 1 Chemical Structure of Daptomycin[19].

2. Development of Daptomycin

The mechanism of biological synthesis of Daptomycin has always remained an inquisitive and interesting phenomenon. Various possible strategies have been applied to increase the production and activity of this useful antibiotic. Literatures suggest that Daptomycin has a peculiar structure consisting of 13 amino acids and shares a ten-membered macrolactone ring and three exocyclic residues. The factors can be distinguished by the fatty acyl-moiety attached to the *N*-terminal Trp_1, which ranges from 10-13 carbon atoms. These fatty acyl moieties comprise of *n*-decanoyl, *anteiso*-undecanoyl, *iso*-dodecanoyl and *anteiso*-tridecanoyl, respectively. The peptide core is composed of a set of nonproteinogenic amino

acids including D-Asn_2, Orn_6, D-Ala_8, D-Ser_{11}, (2*S*, 3R)-methyl glutamate (MeGlu) and kynurenine (Kyn_{13}), that forms an ester bond with Thr_4 and builds up the macrolactone ring[20] . Daptomycin inherits a specific EF-hand motif (DXDG) initially found in the ribosomally assembled calmodulin that is supposed to be involved in Ca2 +-binding [21]. The relative position of D-configured amino acids is conserved within this family as is the long chain fatty acid attached to the cyclic core. The typical biosynthesis of Daptomycin by *S. roseosporus* is governed by three nonribosomal peptide synthetases (NRPS), DptA, DptBC and DptD and *in trans*-acting enzymes [20].

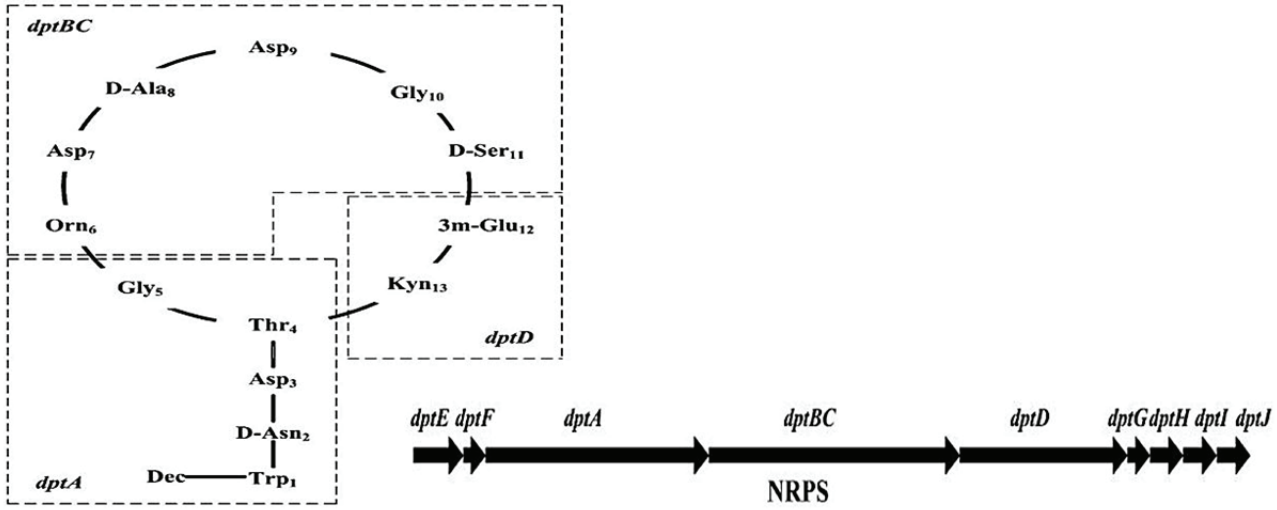

Fig. 2 Representing the Biosynthesis of Daptomycin [19].

2.1 The multidomain organization of Nonribosomal peptide synthetases (NRPS)

Nonribosomal peptides are synthesized by nonribosomal peptide-synthetase (NRPS) enzymes which are independent of messenger RNA. Each module is responsible for the specific recognition, activation, covalent binding and incorporation of a building block into the oligopeptide chain and can be furthermore dissected into catalytic domains [22]. A set of gene is involved in the acylation of the *N*-terminal amino acid. The initiation mechanism consists of an acyl-CoA ligase, that activates the fatty acid, and an acyl-carrier-protein (ACP) to which the FA is covalently bound [20]. The *N*-terminal C-domain (type C^{III}) of the initiation module subsequently catalyzes the condensation between the FA and Trp_1 and chain elongation is commenced[24]. Initiation of daptomycin biosynthesis is mediated by the action of the two distinct enzymes DptE and DptF, encoded upstream of *dptD*[25]. DptE, which shares a high degree of homology to the acyl-CoA ligase superfamily, was shown to activate the fatty acid moiety attached to the *N*-terminus of daptomycin in an ATP-dependent manner. The activated FA is subsequently transferred onto the 4'-Ppan group of DptF, the cognate acyl-carrier-protein (ACP). The *N*-terminal C-domain of the DptA initiation module is predicted to catalyze the condensation of the ACP-bound FA and tryptophan. The broad substrate tolerance of DptE towards the length and type of fatty acids is believed to reflect in the composition of the A21978C factors [7]. After the initiation, chain elongation is mediated by the linearly operating NRPSs DptA, DptBC and DptD. The three epimerization domains present in the synthetases correlate with the D-configured amino acids D-Asn_2, D-Ala_8 and D-Ser_{11}, respectively [20]. Apart from the nonproteinogenic amino acids Orn_6 and Kyn_{13}, a β-methylated glutamate residue ($MeGlu_{12}$) is located within the ten-membered macrolactone ring [26]. Deacylation was achieved by a highly efficient deacylase derived from *Actinoplanes utahensis*[28]. Reacylation was performed chemically with activated acyl esters after protection of side-chain nucleophiles. The generated derivatives varied not only in the acyl functionality but also in the number of exocyclic amino acids (e.g. *α*-N-acylated-Phe)[29].

2.2 Combinatorial Approach for Biosynthesis

Combinatorial approach of biosynthesis of Daptomycin has gained much importance as it involves an entire reprogramming of genes responsible for the enzymatic machinery of the product [32]. *S. roseosporus* can easily accept genetic manipulations and the responsible gene cluster has been sequenced, cloned and heterologously expressed [33] .Combinatorial biosynthesis of daptomycin and hybrid molecules in *S. roseosporus* has already been extensively carried out [27,34-35]. The modular NRPS assembly line offers substitutions and manipulations through tailoring from a single module to multi modules. Initially, *dptA* and *dptD* were deleted from the original locus[36]. These genes were subsequently introduced into *S. roseosporus* to trans-complement deletions of *dptA* and *dptD* by construction of plasmid cloning vectors allowing conjugal transfer of genetic information from *Escherichia coli* to the target strain [36-37].

3. Mode of action of Daptomycin

Linezolid is protein synthesis inhibitor which stops the bacterial growth by disrupting translation of messenger RNA (mRNA) into proteins in the ribosome. It prevents the formation of the initiation complexes as it binds to the 23S portion of the 50S subunit[38]. The intrinsic resistance of most Gram-negative bacteria to linezolid is due to the activity of efflux pumps, which actively "pump" linezolid out of the cell faster than it can accumulate. Vancomycin acts by inhibiting proper cell wall synthesis in gram-positive bacteria[39]. Vancomycin is not active against gram-negative bacteria. The large hydrophilic molecule is able to form hydrogen bond interactions with the terminal D-alanyl-D-alanine moieties of the NAM/NAG-peptides. This binding of vancomycin to the D-Ala-D-Ala prevents cell wall synthesis of the long polymers of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) that form the backbone strands of the bacterial cell wall, and it prevents the backbone polymers that do manage to form from cross-linking with each other. In resistant bacteria, the last D-ala residue has been replaced by a D-lactate, so vancomycin cannot bind. In the nonresistant bacteria, the vancomycin bound to the peptide chains prevents them from interacting properly with the cell wall cross-linking enzyme. However, in the resistant bacteria, stable cross links are formed[40]. The distinctive action Daptomycin in antibacterial activities can be better understood by its mode of action. The present mode of action of Daptomycin suggests the discrete role of calcium ions in the antimicrobial activities . The initial studies by Jung *et al*. proposed a two-step mechanism of action derived from structural changes observed in NMR-experiments, CD-measurements and fluorescence spectroscopy. In the first step Ca^{2+} binds to daptomycin in solution and induces a conformational change, increasing amphipathicity and decreasing its charge [41]. This process facilitates oligomerization and leads to micelle formation which allows daptomycin to interact with neutral or acidic membranes. In a second step, Ca^{2+} bridges the gap between daptomycin and the acidic phospholipids. As indicated by CD-measurements, daptomycin undergoes a second structural transition allowing a deeper insertion into the membrane bilayer.

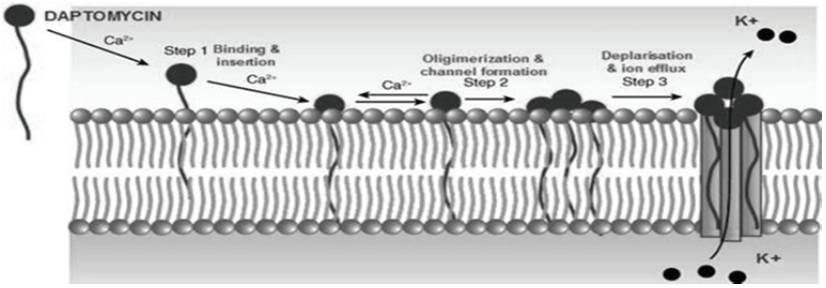

Fig. 3 The mode of action of Daptomycin [41].

In contrast to the study conducted by Silverman *et al*., it was proposed that cytoplasmic membrane depolarization is not the main cause of cell death as it occurred subsequently [42]. The recent studies negate this mode of action on the basis of an NMR-structure of Daptomycin in the presence of Mg^{2+}[43]. It was found that Mg^{2+} also promotes micelle formation, but does not induce a conformational change. Daptomycin was found to form oligomers consisting of 14-16 monomers upon addition of Ca^{2+} in a 1:1-ratio. In accordance to Jung *et al*. it was proposed that divalent cations mask the negatively charged residues and enable micelle formation either by -stacking interactions between aromatic residues or by arrangement of the lipid tails towards the interior of the micelle [43]. Scott *et al.* [44] confirm that Daptomycin experiences only a minor conformational rearrangement upon binding to DHPC in the presence of Ca^{2+}.

4. Production Strategies for Daptomycin

The increased demand of Daptomycin has led to different processing strategies to improve its production. The wild type strain of *Streptomyces roseosporus* NRRL11379 has been preferentially used for Daptomycin production [45]. Also, S. roseosporus LC-51, a mutant of S. roseosporus NRRL11379 has shown improved ability for Daptomycin production. Rational screening based on metabolic engineering is being considered for improvement of the selection process. Low-power laser irradiation technology is being used for high yielding strains, nowadays [46]. Chemical mutation by N-methyl-N-nitro-N nitrosoguanidine (NTG) has been reported as a successful method for mutation and screening of high-yield strains, which can be adapted by any in Pharmaceutical Industry for Antibiotic Production.[47] Various strategies, such as medium optimization, fermentative strategy, genetic engineering modification and metabolic flux analysis have been established to achieve higher production of Daptomycin.

Table 2 Various strains producing substantial amount of Daptomycin.

Strains	Daptomycin or A21978C (mg/L)	Working volume (L)	Culture time (h)	References
Streptomyces lividans TK23 and TK64	55 (A21978C)	–	168–240	Penn et al. [49]
Streptomyces roseosporus NRRL11379	812	3.6	282	I-Son Ng et al. [45]
Streptomyces roseosporus LC-51	632	7.5	132	Yu et al. [48]
Streptomyces roseosporus NRRL11379	296 (A21978C)	7.0	144	Lu et al. [46]

4.1 Role of manipulation of Cofactors for enhanced Daptomycin Production

Biochemical reactions are governed by various cofactors of enzymes which play a key role in the production of various fermentation products.Thus, the manipulation of cofactors' concentration in the fermentation culture could be crucial in order to increase overall process yield of the secondary metabolites [48]. The effects of eight cofactors of enzymes on Daptomycin production were usually investigated through many researches, which included Nicotinic acid (VPP), Riboflavin (VB2), Heme, Thiamine (VB1), Biotin (VH), Cyanocobalamin (VB12), Tetrahydrofolic acid (THF) and Pyridoxal 5-phosphate (VB6). The effects of Heme, THF, VB12 and VB6 on Daptomycin production were especially notable. Daptomycin yield increased to 632 mg/l, which is over 4.5-fold higher than that of the control (without cofactors), at 132 h in a 7.5 Litre fermenter, by supplementation all of the eight cofactors at optimized concentrations (VPP 4 mg/l, VB2 0.5 mg/l, Heme 9 mg/l, VB1 0.4 mg/l, VH 0.1 mg/l, VB12 0.04 mg/l, THF 6 mg/l and VB6 0.4 mg/l). This strategy used for for increasing the Daptomycin production in Streptomyces roseosporus LC-51 by manipulation of cofactors concentration in the fermentation culture may provide an alternative approach to enhance the production of metabolites in other Streptomyces [48, 52].

4.2 Precursor Utilization in Daptomycin Production

Decanoic acid is the essential precursor for Daptomycin production. Almost no daptomycin is produced from S. roseosporus without decanoic derivative chemicals addition. Decanoic acid, sodium decanoate and Cuphea oil [49]which is also a rich source of decanoic acid have been used to improve the Daptomycin fermentation. The two major problems of precursor inhibition and product inhibition of *S.roseosporus* need to be addressed. Many research from literature indicated that the growth of cells would be inhibited by the toxicity of n-decanoic acid by overfeeding of n-decanoic acid during fermentation.[48] Decanoic acid, Sodium decanoate and Cuphea oil were compared for Daptomycin production. However, the decanoic acid (a type of organic acid) might be a kind of the toxic element in culture of *S. roseosporus*.

Table 3 Comparitive Daptomycin production using various precursors.

S.No.	Precursors	Daptomycin Production(mg/L)	Culture Time(h)	Working Volume(L)	References
1	Without any Precursor	Not defined	282	3.6	[45]
2	Decanoic acid	296	144	7	[46]
3	Sodium Decanoate	812	282	3.6	[45]
4	Cuphea oil	600	186	20	[50]

As the precursor, sodium decanoate was in favor of the daptomycin production compared with the aliphatic fatty acids which were examined for enhancement of other lipopeptide antibiotics. The addition time of precursor with maximum daptomycin production was achieved when sodium decanoate was added at the second day after inoculation. Delaying the addition time of the precursor also prevented inhibition of cell growth [51].

4.3 Fed Batch Strategy for Daptomycin Production

Substrate inhibition and product inhibition are two major problems that hamper antibiotic production. In order to have large scale production of antibiotic without losing much product to substrate inhibition, improved fed batch strategy is established as a feedback control. Daptomycin was produced in shake-flasks, batch, and fed-batch fermentation. However, the production of Daptomycin was effectively increased in by intermittent feeding of dextrin upto 812.0 mg/L from 217.5 mg/L in batch fermentation. [45]. Fed-batch strategy developed in fermentation is often applicable in the improvement of antimicrobial products [52].

4.4 Flux Analysis to improve media utilization

A comprehensive metabolic flux analysis model was set up and used to evaluate Daptomycin metabolism, amino acid utilization, and simultaneous consumption of nutrient sources. Stoichiometric model were used to demonstrate the

intracellular carbon flux distribution. This was an innovative approach which compared the flux changes for different fermentation conditions and helped to interpret of the dependency of Daptomycin yield on environmental perturbations (e.g. pH) and principal pathways. Experimental and calculated values for both the specific growth rate of *Streptomyces roseosporus* LC-54-20 (mutated strain) and Daptomycin production rate indicated that the in silico model proved a powerful tool to analyze the metabolic behaviors. Flux distribution variations revealed that the daptomycin production could be significantly influenced by the branch nodes of glucose 6-phosphate, 3-phosphoglycerate, phosphoenolpyruvate, pyruvate, and oxaloacetate. Unlike the previous studies [29], where decanoic acid was the sole precursor which controlled Daptomycin production, the yield of Daptomycin was enhanced by adding five precursors in batch fermentation. [53] The strategy embellished conventional medium design. Many recent researches also suggest the role of metabolic engineering of the genes involved in biosynthesis of Daptomycin in enhancement of carbon uptake and precursor tolerance.

4.5 Total Chemical synthesis of Daptomycin

The first total chemical synthesis of Daptomycin was recently illustrated. Instead of biosynthesis, a complex strategy was developed using a combination of solution-phase synthesis and SPPS to successfully assemble the linear Daptomycin peptide precursor. An efficient and scalable ozonolysis strategy was introduced to synthesize Kyn-containing peptide fragments from the correspondingTrp-containing peptides. The key macrocyclization step in the synthesis of Daptomycin was achieved via a chemoselective serine ligation, which enabled the large-scale synthesis of daptomycin since the cyclization could be accomplished at concentrations of up to 50 mM. Two important conclusions were drawn from this methodology -serine/threonine ligation could be a general tool for peptide cyclization;[54] and ozonolysis of a Trp-containing peptide fragment can lead to the formation of the corresponding Kyn-containing peptide fragment in a highly efficient manner if the Trp moiety is protected. This success suggests that a Kyn-containing cyclic peptide could be readily synthesized via serine/threonine ligation and ozonolysis from a suitable linear Trp-containing peptide precursor.[55]

4.6 Future Considerations for bulk production

The chemical synthesis of key intermediates involves multiple steps and hazardous reaction conditions. To develop high-valued antibiotics at large-scale like Daptomycin, various strategies should be taken up to improve mass transfer characteristics [56-57]. The fermentation process is highly aerobic and involves filamentous microorganism (actinomycetes). Free and immobilized microbial cells can be cultivated using various cultivation modes of batch and continuous strategy using different bioreactors (stirred tank bioreactor, air lift bioreactor and packed bed column)[58-60].

5. Clinical Significance of Daptomycin-Case Studies

5.1 Deep sternal wound infections post cardiac operation

Papov et.al. reported the incidence of deep sternal wound infection (DSWI) after cardiac surgery, 0.4-5% with *Staphylococcus aureus* as the most common pathogen isolated from infected wound sternotomies and bacteraemic blood cultures. This infection is associated with a higher morbidity and mortality than other known causations. The application of Daptomycin was quantified for the treatment of deep sternal wound infection due to gram-positive organisms post cardiac surgery. An observational analysis was carried out for 23 cases of post sternotomy deep sternal wound infection I with gram-positive organisms in February 2009 and September 2010. The incidence of deep sternal wound infection was 1.46%. The mean dose of Daptomycin application was 4.4 ± 0.9 mg/kg/d and the average duration of the Daptomycin application was 14.47 ± 7.33 days. Treatment of deep sternal wound infection due to gram-positive organisms with a Daptomycin-containing antibiotic regimen was found to be safe, effective for local wound infections. [61]

5.2 Diabetic foot ulcers and infections

Total103 patients diagnosed with diabetic foot ulcer infection were clinically evaluated; 47 were treated with Daptomycin and 56 received a comparator. Infections were predominantly due to *Staphylococcus aureus*. Success rates for patients treated with Daptomycin or the comparators were not statistically different for clinical (66% versus 70%, respectively; 95% CI, −14.4, 21.8) or microbiological outcomes. Both treatments were generally well tolerated, with most adverse events of mild to moderate severity. [62]

5.3 Combination drugs containing Daptomycin for Vancomycin-Resistant *Enterococcus faecium*

Fosfomycin is an effective drug for vancomycin-resistant enterococcus (VRE) infections. 32 VRE isolates from renal transplant patients with urinary stent infections were susceptible to fosfomycin, daptomycin, and linezolid and resistant

to amoxicillin, minocycline, and nitrofurantoin based on their MIC50s and MIC90s. Fosfomycin was bacteriostatic at 0.5 to 16_the MIC (32 to 2,048 _g/ml); synergy occurred when fosfomycin was combined with daptomycin (2.8 to 3.9 log10 CFU/ml kill; $P<0.001$) or amoxicillin (2.6 to 3.4; $P<0.05$). These combinations were found to be potent options to treat VRE urinary infections pending investigation of clinical efficacy [63].

5.4 Daptomycin for osteomyelitis caused by MRSA in a renal transplant recipient with FabryAnderson disease

Daptomycin is licensed in adults for the management of *Staphylococcus aureus* methicillinresistantinfections, including bone and skin complicated infections. We describe for the first time its use in a renal transplant recipient for FabryAnderson Disease with right heel osteomyelitis. The patient was unresponsive to firstline Teicoplanin and secondline Tigecycline, whereas he was successfully treated with thirdline Daptomycin monotherapy at 4 mg/Kg/qd for 4 weeks. Local debridement was performed in advance of each line of treatment [64].

5.5 Brain Damage and Hearing Loss in Infant Rat Pneumococcal Meningitis

Aggravation of cerebrospinal fluid (CSF) inflammation in response to bacteriolysis by beta-lactam antibiotics contributes to brain damage and neurological sequelae in bacterial meningitis. Daptomycin, a non-lytic antibiotic acting on Gram-positive bacteria, lessens inflammation and brain injury compared to ceftriaxone. With a view to a clinical application for pediatric bacterial meningitis, the effect of combining daptomycin or rifampin with ceftriaxone was evaluated in an infant rat pneumococcal meningitis model. CSF was sampled at 6 and 22 h after the initiation of therapy and was assessed for concentrations of defined chemokines and cytokines. Brain damage was quantified by histomorphometry at 40 h after infection and hearing loss was assessed at 3 weeks afterinfection. Daptomycin plus ceftriaxone versus ceftriaxone significantly ($P<0.04$) lowered CSF concentrations of monocyte chemoattractant protein 1 (MCP-1), MIP-1_, and interleukin 6 (IL-6) at 6 h and MIP-1_, IL-6, and IL-10 at 22 h after initiation of therapy, led to significantly ($P<0.01$) less apoptosis, and significantly ($P<0.01$) improved hearing capacity. While rifampin plus ceftriaxone versus ceftriaxone also led to lower CSF inflammation ($P<0.02$ for IL-6 at 6 h), it had no significant effect on apoptosis and hearing capacity. Adjuvant daptomycin could therefore offer added benefits for the treatment of pediatric pneumococcal meningitis [65].

5.6 Immunomodulatory effects of Daptomycin.

After vancomycin and linezolid, dapto-mycin gains increasing importance in treatment of wound infections after cardiac surgery. Daptomycin showed immunomodulatory properties, resulting in the suppression of cytokine expression after host immune response stimulation by MRSA. Experimental studies revealed an improved efficacy of daptomycin in combination with administration of vitamin E before infecting wounds by MRSA[66].

5.7 Prosthetic joint infection by Enterococcus faecalis

Enterococci are implicated in less than 2.3% of prosthetic joint infections. These infections can be difficult to treat and therapeutic failures are not uncommon. In these situations, Daptomycin is a safe and effective alternative. This clinical case was presented with a successful response to the prolonged use of high dose Daptomycin[67].

6. Conclusion

The prominence of bactericidal effect of Daptomycin against Gram-positive organisms has opened up new scopes to combat MRSA and fight drug resistance. The future prospects of this outstanding drug are quite bright and promising. It is indeed a miraculous drug that can effectively deal with life-threatening infections, especially drug-resistant staphylococcal infections. Though much endeavor has been made to bring forth the biosynthetic mechanisms of Daptomycin through various literary reports. Yet, the yield of Daptomycin needs to be further enhanced in a cost-effective manner. In order to avail the benefits of this high-value secondary metabolite, new strategies should be incorporated for its large-scale production. The amalgamation of metabolic engineering as well as biochemical engineering would open up new paths for this valuable product. Also, an awareness about this drug and efforts to improve its bulk production would help to serve a larger population.

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