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A Review on Daptomycin; the first US-FDA approved Lipopeptide antibiotics

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Abstract

Antibiotic resistance among Gram-positive bacteria, particularly in *Staphylococcus* spp., *Enterococcus* spp. and *Streptococci* Spp., continues to increase and has become a major clinical problem. The need for new antimicrobials active against these bacteria has now been partly met by the appearance of a number of new compounds with excellent activity against multiple-resistant Gram-positive Cocci. Daptomycin is a novel pharmaceutical molecule produced by Streptomyces roseosporus, which was discovered in late 1980's, has demonstrated a broad spectrum of activity in vitro against a wide range of aerobic and anaerobic gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci. Daptomycin has a unique mechanism of action, not completely understood, involving a calcium-dependent dissipation of membrane potential leading to the release of intracellular ions from the cell and bacteria death. This antibiotic has got the FDA approval only in 2003 for the treatment of patients with complicated skin and skin structure infections, right-sided endocarditis and bacteraemia.

Keywords: Gram-positive bacteria, Daptomycin, *Streptomyces roseosporus*, Calcium-dependent.

Introduction

The increase in infections caused by Gram-positive pathogens and the rise in antibiotic-resistant bacterial strains have prompted the need for novel antibiotics.[1, 2] Recent reports indicate that more than 25% of *Staphylococcus aureus* infections in Europe are caused by methicillin-resistant *S. aureus* (MRSA), and the majority of these isolates are resistant to additional antibiotics.[2] The incidence of MRSA varies greatly by country. Over 50% of S. aureus isolates in Portugal and Italy are methicillin-resistant, isolates in England, Greece, and France have MRSA rates around 25%, whereas the Netherlands and Switzerland have the lowest incidence of MRSA.[3] The Centers for Disease Control and Prevention (CDC) estimate that the number of people developing a serious MRSA infection in 2005 (in USA), was about 94,360. The number of people is also remarkable that died during hospital stays related to these serious MRSA infections, approximately 18,650. For many years, MRSA remained a problem restricted to hospitals, intensive care units and other health care medical facilities.[4, 5] Vancomycin has been an effective antibiotic against MRSA; however, the increased use of vancomycin has led to the development of isolates with reduced susceptibility. The mechanism of reduced susceptibility to vancomycin in *S. aureus* has not been fully elucidated and appears to be heterogeneous.

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Reduced susceptibility to vancomycin is correlated with alterations in the bacterial cell wall leading to significantly thicker and more disorganized cell walls.[6] These thicker cell walls may sequester the vancomycin from reaching the target nascent cell wall precursors. Therefore, the need for new potent antimicrobial agents with MRSA activity is essential.[7]

Daptomycin is a natural product of soil actinomycetes, as are most of the important antibiotics developed in the past 50 years Daptomycin is the first approved member of the A21978C family of the cyclic anionic 13-amino acid lipopeptide, produced as a secondary metabolite by Streptomyces roseosporus antibiotics - the cyclic lipopeptides. Daptomycin 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,[8-9] but was ultimately shelved due to concerns regarding adverse effects, particularly drug induced myopathy.[10] Cubist Pharmaceuticals Inc. licensed worldwide rights from Eli Lilly and Company in 1997.[11] After an intensive role of clinical trials, the U.S. Food and Drug Administration (FDA) approval was obtained in 2003.[9]

Figure 1: Representing the Chemical Structure of Daptomycin

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.

Daptomycin, which contains an n-decanoyl side chain, was chosen for clinical development because of its in vivo efficacy and low toxicity in animals.[12] Because enzymatic de-acylation, coupled with chemical re-acylation with decanoate, was not cost-effective to manufacture, scientists

at Eli Lilly developed a process that fed decanoic acid during fermentation, to directly produce Daptomycin.[10, 13] Supplementation of cofactors in central metabolic pathway enhanced glycolysis and citric acid cycle. Thus, more carbon skeleton and ATP could be offered for the biosynthesis of the Daptomycin precursors.[14] The high water solubility of Daptomycin is due to its predominantly acidic nature and the negative charge (3-) at neutral pH. Its lipid tails and some hydrophobic amino acids warrant amphipathic properties.[15]

Daptomycin, a Prototype of the Acidic Lipopeptide Family

Daptomycin, the first approved lipopeptide antibiotics, is a member of the A21978C family of the cyclic anionic 13-amino acid lipopeptide, Produced by precursor of n-decanoic acid-directed fermentation as a secondary metabolite by Streptomyces roseosporus (Fig.2).[16] Daptomycin is produced by a non-ribosomal peptide synthetase (NRPS) mechanism in S. roseosporus. The A21978C factors composed of 13 D- and L- amino acids and share a 10-member macrolactone ring and three exocyclic residues. The factors can be distinguished by the fatty acyl moiety attached to the N-terminal Trp1, which ranges from 10 to 13 carbon atoms. These fatty acyl moieties comprise n-decanoyl, anteiso-undecanoyl, iso-dodecanoyl, and anteiso-tridecanoyl, respectively (Fig. 1). The peptide core is composed of a set of non-proteinogenic amino acids, including D-Asn2, Orn6, D-Ala8, D-Ser11, (2S,3R)-methylglutamate (MeGlu), and kynurenine (Kyn13), that forms an ester bond with Thr4 and builds up the macrolactone ring.[15]

Daptomycin has been commercialized for the treatment of skin and skin structure infections caused by Gram-positive pathogens.[17, 18] Daptomycin is synthesized by a large non-ribosomal peptide synthetase multienzyme (NRPS), encoded by three very large genes, dptA, dptBC, and dptD arranged in tandem (Fig. 3) each subunit contains two to six modules to direct the incorporation of the 13 amino acids. The NRPS up stream genes of dptE and dptF show sequence similarities to acyl-CoA ligase and acyl carrier protein genes, respectively. DptE and DptF are predicted to be involved in the acylation of the N-terminal tryptophan (Trp), which initiates the biosynthesis of Daptomycin. The group of contiguous genes, including dptG, dptH, dptI, and dptJ, located downstream of the NRPS genes also have important functions in the biosynthesis of daptomycin. Comparative domain analyses suggest involvement of dptG in regulation of expression or export of antibiotics.
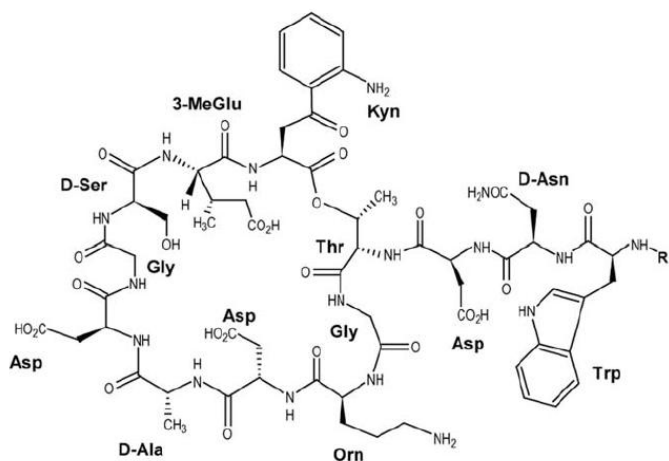
While dptH may enhance the efficiency of daptomycin production by clearing misincorporated substrates that block the pathway. The dptJ encodes the tryptophan 2, 3-dioxygenase, an enzyme involved in the conversion of Trp to kynurenine. The dptI is involved in the formation of 3-methylglutamic acid in vivo.[14]
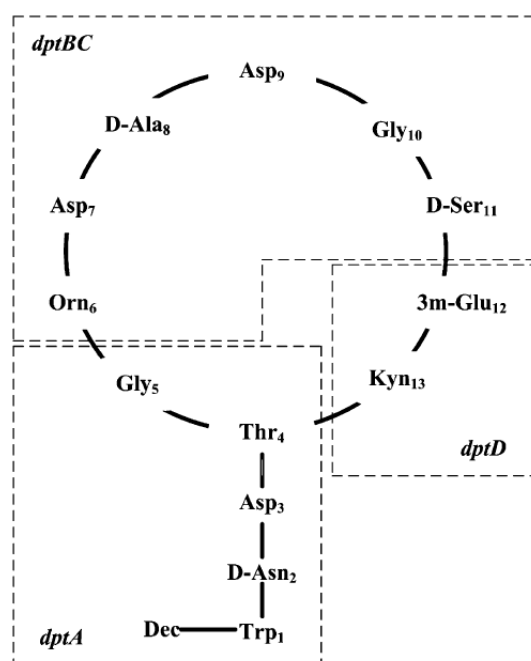

Figure 2: Representing the Biosynthesis of Daptomycin Precursor

Figure 3: Representing the Main Part of the Dpt gene Cluster

Mechanism of Action

Daptomycin, a fermentation product produced by Streptomyces roseosporus, is a cyclic lipopeptide antibiotic with potent bactericidal activity against most Gram-positive organisms including multiple antibiotic-resistant and susceptible strains. Daptomycin was recently approved in the United States for the treatment of complicated skin and skin structure infections (cSSSI) associated with *S. aureus* (methicillin-susceptible, MSSA, and methicillin-resistant, MRSA), Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus dysgalactiae subsp. Equisimilis and Enterococcus faecalis (vancomycin-susceptible only). The unique structure of Daptomycin consists of a 13-member amino acid cyclic lipopeptide with a decanoyl side-chain (Figure 1). This distinctive structure confers a novel mechanism of action.[19, 20]

A two-step mechanism of action derived from structural changes observed in NMR experiments, CD measurements, and fluorescence spectroscopy.[21] In the first step, Ca2+ binds to daptomycin in solution and induces a conformational change, increasing amphipathicity and decreasing its charge. This process facilitates oligomerization and leads to micelle formation, which allows daptomycin to interact with neutral or acidic membranes. In a second step, Ca2+ 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.[22]

Daptomycin inserts directly into the cytoplasmic membrane of Gram-positive cells (aerobes and anaerobes). This action is calcium-dependent and the mechanism involves insertion of the lipophilic Daptomycin tail into the bacterial cell membrane, causing rapid membrane depolarization and a potassium ion efflux. This is followed by arrest of DNA, RNA and protein synthesis resulting in bacterial cell death (Figure 4).[23-24] 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 bactericidal effect of Daptomycin is rapid with greater than 99.9% of both MRSA and MSSA bacteria dead in less than 1 h.[24, 25] Daptomycin also remains bactericidal (99.9% kill within 24 h) against stationary phase cultures of both MSSA and MRSA present at high density (109 cfu) in a simulated endocardial vegetation model.[20]

Daptomycin is also a very promising agent in inhibiting organisms embedded in biofilm. It showed that fluorescently tagged Daptomycin accessed the interior of S. epidermidis biofilm cell clusters within minutes. Structurally, biofilms are multi-layered cell clusters embedded in a matrix of extracellular polysaccharide (slime).[26-28] Microorganisms growing in biofilms are significantly more resistant to killing because of much better protection against macrophages and antibiotics than their planktonic (free- living) counterparts. Several in vitro studies show that Daptomycin alone or in combination with other antibiotics like Rifampin is an important candidate for prevention and treatment of staphylococcal biofilm-related infections.[26]
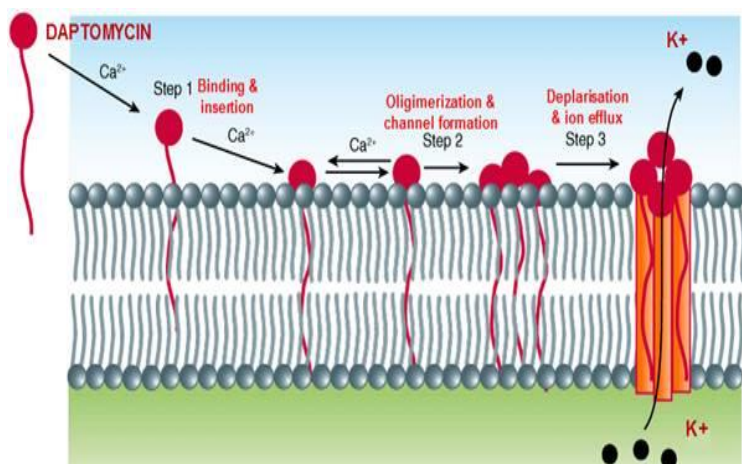
Figure 4: Representing Mechanism of Action of Daptomycin

Fermentative Production

Daptomycin is produced by 2 different manufacturing Processes Steps, includes: fermentation i.e. inoculum preparation, fermentation and purification by chromatography, ultrafiltration, and filling in low-density polyethylene (LDPE) bioprocess container. Characterisations of impurities are conducted based on detailed evaluation of Daptomycin impurity profile. The main impurities include 3 fermentation process related impurities and three degradation products namely Anhydro-Daptomycin, ß-aspartyl Isomer and Lactone Hydrolysis product of Daptomycin. Changes are made to the purification steps and to the final presentation of Daptomycin (frozen concentrate instead of lyophilised powder). Comparative analytical results demonstrate that

Daptomycin batches used in pre-clinical and clinical studies produced according to the current and the earlier processes are physicochemically equivalent.[10, 29]

Materials and methods

Almost all antibiotics have product inhibition effects due to the precise and rigorous regulation and insufficient resistance to self-production antibiotics. Thus, the strains with enhanced resistance to self-production antibiotics often have improved antibiotics productivity. Further, 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.[14]

Microorganism and Spores Suspensions

The wild type strain of Streptomyces roseosprus NRRL11379 is mentioned in the literature for Daptomycin production. It is found that S. roseosporus LC-51 is a mutant of S. roseosporus NRRL11379, screened by laboratory, which can utilize inorganic compounds as the sole nitrogen sources and has improved ability of Daptomycin production can be used as a wild-type and parent strain. S. roseosporus LC-51 culture stocks are maintained in 15% glycerol at −70 °C. Spores on slant are inoculated into 30 mL of vegetative medium in a 250-mL shake flask and incubated in an orbital shaker at 30 °C and 220 rpm for 48 h. Spores suspension harvested then filtrated and can be counted microscopically (usually about 108 spores/mL).[14]
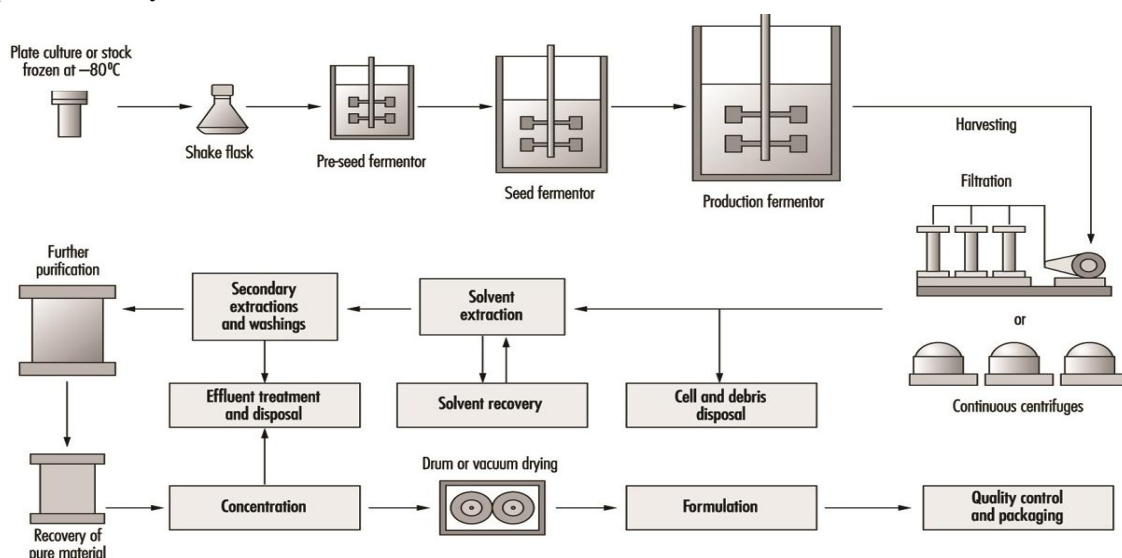

Figure 4: Representing General Flow Diagram for Antibiotic Production in Pharmaceutical

The modern techniques of genetic engineering and metabolic engineering are considered to be effective methods in the microorganism breeding. Traditional

mutation is still an effective approach to obtain improved strains for overproduction of both primary and secondary metabolites.[22] Rational screening, on the basis of

understanding the metabolism and pathway regulation, allows for a significant improvement in the efficiency of the selection process, by contrast with the time-consuming random selection method. Moreover, precursor-resistant mutants and product-resistant mutants are widely used for screening high producing strains. Low-power laser irradiation technology has brought great interest in the microorganism mutation breeding nowadays.[15] 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.[10]

Medium and culture conditions

The slant medium (g/l):

Starch = 20; KNO3 = 1; MgSO4.7H2O = 0.5; NaCl = 0.5; K2HPO4.3H2O =0.5 and Fe2 (SO4)3 =0.1, dissolved in 1 Litre distilled water and the pH adjusted to 7.5.

The seed medium A (g/l):

Glucose = 5; Peptone = 5; Yeast Extract = 8; Dextrin = 15; Peanut Powder = 5; K2HPO4.3H2O =0.5; MgSO4.7H2O = 0.5 and CaCO3 =0.2, dissolved in 1 Litre distilled water and the pH adjusted to 7.5.

The fermentation medium B (g/l):

Glucose =60; KNO3 =2; (NH4)2SO4 = 2; NH4 NO3 =5; K2HPO4.3H2O = 0.5; MgSO4.7H2O =0.5 and CaCO3 = 0.2, dissolved in 1 Litre distilled water and the pH adjusted to 7.5.

Cofactors are added to the sterile culture medium after aseptic filtration. Spores on slant are inoculated into 30 ml of seed medium A in a 250-ml shake flask and incubated in an orbital shaker at 300C, 220 rpm for 48 h. Then, the culture is inoculated into fermentation medium B with an inoculum size of 2% (v/v). Fermentations in a Lab Scale are carried out in 500-ml flasks containing 50 ml medium B, or in a 7.5-Litre with medium B. The flask cultures are incubated at 300C, 220 rpm for 6 days. As for the fermentor cultures, the pH is automatically controlled at 7.0 with 8 M NaOH solution and incubated at 300C, with agitation of 300–400 rpm and the aeration speed of 0.8 v/v/ m for 6 days. N-decanoic acid is fed at 48 h after inoculation (0.2 g/l).[14, 18]

Key point for Designing fermentation

Cofactors of enzymes play an essential role in a large number of biochemical reactions and the production of various fermentation products. It is conceivable that in cofactor-dependent production systems, cofactor availability and the proportion of cofactor in the active form may play an important role in effecting the overall process yield of the metabolites. Thus, the manipulation of cofactors concentration in the fermentation culture could be crucial in order to further increase production of both primary and secondary metabolites.[14, 17]

The effects of eight cofactors of enzymes on Daptomycin production are 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 dry cell weight (DCW), consumption of glucose, and Daptomycin production can obviously be improved when proper amount of exogenous cofactors were supplemented in the medium. The effects of Heme, THF, VB12 and VB6 on Daptomycin production are especially notable. The Daptomycin yield enhanced 363, 104, 53 and 46%, respectively, when optimized amount of these four cofactors were supplemented in the broth. Moreover, the Daptomycin yield further 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). Further, the effects of cofactors on the corresponding key enzymes and important intracellular metabolites are also studied in literature in order to elucidate the mechanism of enhancement of Daptomycin production by manipulation of cofactors concentration in the fermentation culture. It is suggested that this strategy 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.[14]

Downstreaming

Downstreaming is very important aspect in any process industry. Especially in Pharmaceutical industries, special attention is always given to Downstreaming aspects. About 60 % of the total Cost allotted to any product, is given to Downstreaming, which could reaches to 70% in case of Antibiotics or any other Pharmaceutical product. The

Downstreaming involves extraction of as much of product as possible from the fermentation broth with the means of different instruments or modules. The instruments usually involve different types of Filters, Centrifugers, Cell Harvesting Units, Extraction units, etc. Different Solvent extraction methods are available in literature and some of them are actually carried out in the industry, which are usually accepted for Daptomycin production as it is.[14]

Product purification is also very important task in antibiotic fermentation. Many sophisticated units are required which majorly includes HPLC columns, and product concentration units, where maximum purity i.e. about up to 90 % purity is tried to achieve. The broth Concentration of Daptomycin is usually found to be 334 mg/lit which can be further improved up to 632 mg/ lit by addition of proper mixture of Co-factors and with proper downstraming.

Product Specification

The finished product specification include tests for appearance, identification (UV and FTIR), pH, assay (HPLC), powder fill weight, degradation products, uniformity of content, water content, bacterial endotoxins, particulate contamination, sterility, container closure integrity and reconstitution time. The degradation products limits have been satisfactorily justified based on toxicology studies. Satisfactory batch analysis data have been provided for full-scale batches manufactured at industry.[20]

Specification

The active substance specification includes tests for appearance, identity (UV and FTIR), assay (HPLC), impurity content (HPLC), residual solvents (GC), pH, bacterial endotoxins, microbial limits, specific rotation, heavy metals and residue on ignition. The impurity limits are usually satisfactorily justified based on toxicology studies.[20]

The frozen active substance yields a clear, dark yellow to light brown solution upon thawing. X-ray diffraction studies indicated that Daptomycin powder is amorphous. It is highly soluble in water. Stress stability studies showed that it degrades when exposed to direct light, heat, oxygen and to extreme pH in solution.

Pharmacology

The primary pharmacology studies focused on in vitro microbiological profiling, animal models of infection, and pharmacodynamics studies correlating efficacy with pharmacokinetic parameters.

Safety pharmacology

Safety pharmacology was primarily investigated in rodents, dogs, and in vitro models. No adverse effects on the cardiovascular, respiratory, renal, gastrointestinal or immune systems were observed in vivo at clinically relevant doses. Daptomycin's effects on the central nervous system as well as the neuromuscular system were assessed in a series of in vivo and in vitro studies. Effects on the nervous and/or muscular system were evident at high dose intravenous (IV) levels in rodents ($\geq$ 50 mg/kg). Marked effects on the central nervous system in animals were observed only at IV dose levels of $\geq$ 150 mg/kg. At doses $\geq$ 200 mg/kg in mice, tremors and clonic convulsions were observed; with death occurring at $\geq$ 1000 mg/kg. No corresponding effects were observed in rats at 150 mg/kg.[30]

No effect on EEG or neuromuscular conduction was noted in dogs after acute administration of Daptomycin at IV dose levels of 50 and 30 mg/kg, respectively. Daptomycin appears to have no clinically relevant pharmacological activity in cardiac or smooth muscle. In vitro, at high concentrations (up to 25-fold peak plasma concentration observed in patients at intended clinical dose of 4 mg/kg/day), no effects were seen in cardiac (atria) or smooth muscle preparations (ileum, jejunum, or aorta). Effects were limited to selective antagonism of rat uterus and vas deferens smooth muscle preparations. However, the concentration at which these effects were observed (160 μg/ml free drug) is 30-fold greater than the maximal concentration of free drug in patients at the intended clinical dose of 4 mg/kg/day.[31]

Daptomycin is the active ingredient in Cubicin. Cubicin is supplied in a single-use 10 mL vial, as a sterile, preservative-free, pale yellow to light brown, lyophilised cake containing at least 900 mg/g of daptomycin for IV use following reconstitution with 0.9 % sodium chloride injection. Daptomycin has a high aqueous solubility (> 1 g/mL). The only inactive ingredient is sodium hydroxide, which is used in minimal quantities for pH adjustment.[23]

Pharmacokinetics

Single and multiple dose pharmacokinetics studies were conducted in mice, rats, beagle dogs, and rhesus monkeys using both unlabelled and radiolabelled Daptomycin. As the intended clinical route is intravenous, the majority of

the studies use this route of administration. Toxicokinetic studies were included in the 6-month rat study (Day 1 only) and in all repeat dose studies in dogs (up to 6-months) and in monkeys (1-month). Published literature on Daptomycin provided supportive data, as well as information on pharmacokinetics in other species, such as rabbits and guinea pigs, and information on serum protein binding. Methods of analysis were adequate.[31]

Absorption- Bioavailability

Daptomycin shows poor oral bioavailability and can only be administered parenterally.[32] Dosing is based on the body weight of the patient. Overall, the pharmacokinetic characteristics were generally comparable across the 4 species tested. Studies in rats showed that Daptomycin was poorly absorbed orally and exhibited slow passage through the gastrointestinal tract with > 90% excreted in the faeces.[31] Limited data indicated a relatively high bioavailability after SC or IP administration. Daptomycin plasma profile following IV injection was consistent with a 2-compartement model with a rapid distribution phase and slower elimination phase. Daptomycin exhibited linear kinetics following intravenous injection across a dose range of 1 to 50 mg/kg in the rat, 1 to 200 mg/kg in the dog, and 1 to 25 mg/kg in the monkey. For all species, plasma clearance, volume of distribution, and terminal half-life were dose-independent over the linear range. Within the linear range, terminal half-life of Daptomycin was 1 – 3 hours in rodents and 2 – 4 hours in non-rodents. The pharmacokinetic profile of Daptomycin was similar between strain and gender and was not altered upon repeated daily administration for up to 6 months.[27]

Distribution

Daptomycin has a very small volume of distribution of approximately 0.1 L/kg, one of the smallest volumes reported for any drug. This small volume indicates that there is very little tissue distribution and the majority of the drug remains in the blood stream and extracellular fluid space. The reason for the low volume of distribution is the drug's high plasma protein binding that has been reported to be 90 %.[31] This high plasma protein binding always needs to be considered when comparing Daptomycin plasma concentrations with their respective MIC values. Microdialysis is an appropriate technique to monitor the unbound, active drug concentrations in tissues.[30]

In the rat, the only species in which tissue distribution was studied, where Daptomycin distributes rapidly from the plasma to the tissues with a distribution phase half-life ($t\frac{1}{2}$

α) of approximately 7 minutes. The volume of distribution was essentially the same (approximately 60 to 160 ml/kg) in mice, rats, dogs, monkeys and humans, and clearance scales to body size. Daptomycin appears to distribute preferentially to the kidneys, reflecting the vascularisation of the tissue as well as renal concentration of the drug during excretion. In general, the half-life in tissues was slightly greater than that in plasma and tissue levels were higher after repeated dosing than after a single dose. No accumulation was observed in plasma upon repeated administration in rats, dogs, or monkeys. There is no data on the excretion of Daptomycin into milk. Published data indicated that the extent of protein binding was the same in mice, rabbits and humans (approximately 90%).[31]

Excretion

The half-life of Daptomycin is approximately 8 hours the reason for this long half-life is the restricted glomerular filtration due to the high protein binding on the other hand, this long half-life facilitates the possibility of a once-daily dosing regimen.[32] In mice, rats, dogs, monkeys most of the compound ($\geq$ 70 %) was recovered in the urinary within 48 hours post-dose. Faecal excretion accounted for approximately 3 to 10 % of the administered radioactivity in these species. This is comparable to the human data. As expected, patients with impaired renal function show longer half-lives and require longer dosing intervals.[28]

In rats less than 2 % of the administered radioactivity was recovered in the expired air. A study in juvenile dogs showed that total systemic clearance appears to be faster in juvenile dogs as compared to adults, resulting in shorter terminal half-life, at the same dose level. In rats with renal impairment, the systemic clearance was reduced by ~70 % compared to that of normal rats. This resulted in a ~1.5 to 2-fold increase in peak plasma concentration (Tmax), and increased half-life. Volume of distribution was decreased by 53 % .[30, 31]

Daptomycin is a non-typically used natural lipopeptide antibiotic approved by the Food and Drug Administration in 2003 for the treatment of skin and skin structure infections caused by Gram-positive pathogens and for the treatment of bacteraemia and right-sided endocarditis caused by *S. aureus* strains and MRSA in 2006. Daptomycin's approval was based on the result of two randomized, multicentre, investigator-blinded trials comparing Daptomycin to either Vancomycin or Pencillinase-resistant Penicillin (PRSP) in the treatment of complicated skin and skin structure infections (cSSSI).

Clinical trials are on-going examining Daptomycin's efficacy in the treatment of complicated urinary tract infections, bacteraemia, and endocarditis. Trials in endocarditis and bacteraemia are utilizing a higher dose (6 mg/kg IV q24h) than the currently approved dose.

Table 1: Comparison of different properties of two different drugs with Daptomycin (Adapted from 30)

	Vancomycin	Linezolid	Daptomycin
Approved Indications (FDA)	Serious or severe infections caused by susceptible strains of methicillin-resistant (beta-lactam-resistant) staphylococci. Alone or in combination with an aminoglycoside for endocarditis caused by *S. viridans* or *S. bovis*. For endocarditis caused by enterococci (e.g., *E. faecalis*) only in combination with an aminoglycoside.	Vancomycin-Resistant *E. faecium* infections Nosocomial pneumonia caused by *S. aureus* or *S. pneumoniae* including multi-drug resistant strains Complicated skin and skin structure infections, including diabetic foot infections Uncomplicated skin and skin structure infections caused by MSSA or *S. pyogenes*. Community-acquired pneumonia caused by MSSA	Complicated skin and skin structure infections. *S. aureus* bloodstream infections (bacteremia), including those with right-sided infective endocarditis, caused by methicillin-susceptible and methicillin-resistant isolates.
Oral Bioavailability	Not absorbed	Not absorbed	Completely absorbed
Clearance	0.06 L/h/kg	0.01 L/h/kg	0.10 L/h/kg
Volume of Distribution	0.3 to 0.43 L/kg	0.1 L/kg	0.7-0.8 L/kg
Half-Life	4-6 h	8 h	4-5 h
Protein Binding	55 %	90-93 %	31 %
Major Route of Elimination	renal	renal	metabolism 30 % renal
Tissue Penetration	moderate	low	high
Usual Dosing Regimen	1000 mg IV over 60 min. Q12h with Drug Level Monitoring	4-6 mg/kg IV over 30 min. Q24h	600 mg IV over 30-120 min. Q12h 400-600 mg PO Q12h
Dosing in Renal Impairment	Increased Dosing Interval with Drug Level Monitoring	Increased Dosing Interval	No dose adjustment

Conclusion

Daptomycin is a bactericidal antibiotic with outstanding activity against Gram-positive organisms. The market prospect of Daptomycin is bright and promising and the demand of Daptomycin has also been increasing. More importantly, physicians now have an excellent treatment option for life-threatening infections, including increasingly drug-resistant staphylococcal and other infections, which cause thousands of deaths each year. However, the yield of Daptomycin needs to be further enhanced to reduce the cost of industrial production which is now $129.05 for each 500mg vial. The cost reduction of this high-value secondary metabolite is hampered by their poor production; therefore, various disciplines such as Genetics, Physiology, Biochemistry and Biochemical engineering should combine together for a work, which need to be carried out to enhance the production of the antibiotics. Due to the heavy and tedious traditional work for strain screening, it is necessary to explore time and labour saving approaches for production improvement. Constraints-based metabolic flux analysis (MFA) is one of the most popular computational methods for predicting engineered gene targets rationally, which is based on a stoichiometric metabolic model that represents the mass balance information of metabolites in all cellular reactions. Although, to date, little attempt has been made to understand quantitatively the production of Daptomycin by S. roseosporus using the MFA method.

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