Available online at www.sciencedirect.com
SCIENCE DIRECT
Pulmonary Pharmacology & Therapeutics 16 (2003) 131–145
PULMONARY PHARMACOLOGY & THERAPEUTICS
www.elsevier.com/locate/ypupt

What's in the pipeline?

Novelties in the field of antimicrobial compounds for the treatment of lower respiratory tract infections

Mario Cazzola[a,*], Alessandro Sanduzzi[b], Maria Gabriella Matera[c]

[a]Department of Respiratory Medicine, Unit of Pneumology and Allergology, A. Cardarelli Hospital, Via del Parco Margherita 24, 80121 Naples, Italy
[b]Department of Clinical and Experimental Medicine, Unit of Respiratory Medicine, University Federico II, Naples, Italy
[c]Department of Experimental Medicine, Unit of Pharmacology, Second University of Naples, Naples, Italy

Received 23 May 2002; revised 10 February 2003; accepted 11 March 2003

Abstract

Emergence of antimicrobial resistance is a growing problem and a public health threat. New drugs must be designed with emerging needs in mind: specific resistant and hard-to-treat organisms. But the difficulty to find real new drugs is a major problem. Only the oxazolidinones, the cationic peptides and the lipopeptide antibiotics can be truly regarded as structurally novel drugs, although the peptide deformylase inhibitors and, possibly, the pleuromutilins can be considered a potential advancement in the field. Obviously, these antibiotics must be reserved only to cases of documented ineffectiveness of the common antimicrobial agents.

Keywords: Oxazolidinones; Cationic peptides lipopeptides; Peptide deformylase inhibitors; Pleuromutilins

1. Introduction

From the 1980s onwards, resistance has steadily compromised standard therapy of bacterial lower respiratory tract infections (LRTIs). The spread of methicillin-resistant staphylococci, and penicillin- and macrolide-resistant pneumococci, the emergence of glycopeptide-resistant staphylococci, and the increasing emergence of erythromycin-resistant strains of *Streptococcus pyogenes* underline the need for therapeutic alternatives. Although the rational use of antibiotics can limit the development of resistances, it is not sufficient to abate the resistant bacteria. Therefore, new drugs must be designed with emerging needs in mind: specific resistant and hard-to-treat organisms. But the difficulty to find real new drugs is a major problem. In effect, only the oxazolidinones, which have recently been launched in several countries, the cationic peptides and the lipopeptide antibiotics can be truly regarded as structurally novel drugs, although the peptide deformylase inhibitors and, possibly, the pleuromutilins can be considered a potential advancement in the field. The other agents that are under development or have just been marketed are analogues of existing compounds, which have been in use for many years [1].

2. Oxazolidinones

DuPont researchers reported the first oxazolidinones in 1987 that had limited activity against *Mycobacterium tuberculosis* [2]. Introduction of the fluorine substituent afforded compounds with excellent activity against resistant Gram-positive cocci. They possess a unique mechanism of bacterial protein synthesis inhibition [3]. Cell-free transcription/translation systems from *Escherichia coli* were used to demonstrate that their activity as inhibitors of translation decreased as the amount of mRNA (phage MS2 RNA) in the assay was increased. These data indicate that the mechanism of action of this class of drugs involves interference with the binding of mRNA to the ribosomes at the initiation phase of translation. Oxazolidinones inhibit initiation of protein synthesis by binding to the 50S ribosomal subunit and preventing it from complexing with the 30S-subunit, mRNA and initiation factors; this stage of protein biosynthesis has not been exploited previously as an antimicrobial target [4]. In particular, they arrest the formation of the initiation complex in bacterial translation

* Corresponding author. Tel.: +39-081-7473334; fax: +39-081-7473331.
E-mail address: mcazzola@qubisoft.it (M. Cazzola).

1094-5539/03/$ - see front matter
doi:10.1016/S1094-5539(03)00050-6

systems by preventing formation of the *N*-formylmethionyl–tRNA-ribosome–mRNA ternary complex. The susceptibility of Gram-positive organisms to oxazolidinones can also be attributed to a lack of Gram-positive transmembrane pumps with oxazolidinone specificity.

2.1. Antibacterial spectrum

The most advanced agents in this class, such as eperezolid (formerly U-100592) and linezolid (formerly U-100766), have bacteriostatic activity against a number of important Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), and penicillin-resistant *Streptococcus pneumoniae* (PRSP), although a bactericidal effect has been described for strains of *S. pneumoniae*. They present a uniform susceptibility in sensitive bacteria that is independent of resistance to other antibiotics. Extremely rare resistance has been associated with amino acid substitutions in the 23S rRNA. They are also active against the atypical bacteria. Both eperezolid and linezolid appear to be efficacious and well tolerated both orally and parenterally at doses that produce plasma concentrations in excess of the levels that are predicted to be necessary for efficacy [5]. Linezolid has superior pharmacokinetics to eperezolid, and its development has been continued. In particular, linezolid demonstrated 100% bioavailability after oral dosing [6].

Linezolid shows good activity against MRSA and coagulase-negative staphylococci, with MIC_{90}s of 4 mg/l and no differences in activities against methicillin-resistant and -susceptible strains. Interestingly, all teicoplanin-resistant coagulase-negative staphylococci are inhibited by 2 mg/l of linezolid (Table 1). The in vitro activity of linezolid against *S. pneumoniae* appears to be independent of penicillin and cefotaxime resistance, and in any case the MICs of linezolid are >2 mg/l. Linezolid also exhibits excellent activity against erythromycin-resistant *S. pneumoniae*. This effect suggests a potential therapeutic option for the treatment of infections due to multiply resistant pneumococci [7]. It must be highlighted that neither the presence of modifying enzymes (LinA, LinA′, LinB, Vgb, Vat, SatA, ANT(4′) (4″)-I, AAC(6′)-APH(2″), APHA-3 and

Table 1
In vitro activity of linezolid compared with that of vancomycin, teicoplanin and other antimicrobial agents against 450 Gram-positive clinical isolates. Adapted from Cercenado et al. [7]

Organism(s) and type (no. tested)	Antimicrobial agent	MIC (mg/l)			%[a]			
		MIC_{50}	MIC_{90}	Range	susc.	interm.	res.	≤4 mg/l
S. aureus methicillin susceptible (31)	Linezolid	2	2	1–2				100
	Vancomycin	1	2	0.5–2	100			
	Teicoplanin	0.5	2	0.12–4	100			
S. aureus methicillin resistant (50)	Linezolid	2	2	0.5–4				100
	Vancomycin	1	2	0.5–4	100			
	Teicoplanin	2	2	0.25–2	100			
Coagulase-negative *Staphylococcus* methicillin susceptible (28)	Linezolid	1	1	0.25–2				100
	Vancomycin	1	2	0.25–2	100			
	Teicoplanin	0.5	2	0.03–4	100			
Coagulase-negative *Staphylococcus* methicillin resistant (46)[b]	Linezolid	0.5	2	0.5–4				100
	Vancomycin	2	2	0.5–4	100			
	Teicoplanin	2	64	0.06–64	67		33	
S. pneumoniae penicillin susceptible (19)	Linezolid	1	1	0.5–2				100
	Vancomycin	0.25	0.25	0.5	100			
	Teicoplanin	≤0.01	≤0.01	≤0.01	100			
	Erythromycin	≤0.25	≤0.25	≤0.25– >4	90			
	Cefotaxime	≤0.06	≤0.06	≤0.06	100	10		
	Penicillin	≤0.03	0.06	≤0.03–0.06	100			
S. pneumoniae penicillin intermediate (27)	Linezolid	0.5	1	0.25–1				100
	Vancomycin	0.25	0.25	0.5	100			
	Teicoplanin	≤0.01	≤0.01	≤0.01	100			
	Erythromycin	4	>4	≤0.25– >4	41		59	
	Cefotaxime	0.5	1	≤0.06–1	81	19		
	Penicillin	0.5	1	0.12–1		100		
S. pneumoniae penicillin resistant (45)	Linezolid	0.5	1	0.12–1				100
	Vancomycin	0.25	0.5	0.5	100			
	Teicoplanin	≤0.01	≤0.01	≤0.01–0.12	100			
	Erythromycin	>4	>4	>4	27	4	69	
	Cefotaxime	1	2	0.5–2	13	53	34	
	Penicillin	4	4	2–4			100	

[a] Percentages of isolates that were susceptible (susc.), intermediate (interm.) and/or resistant (res.) to the agent or inhibited with ≤4 mg/l of the agent.
[b] Includes 15 teicoplanin-resistant strains.

Cat), nor the presence of an efflux mechanism (*MsrA*, *MefE*, *MefA*, *MreA*, *Vga*, *TetK* and *TeL*), nor the modification or protection of antimicrobial target (because of ribosomal methylases or *TetM* and *TetO*) affect linezolid activity as demonstrated by similar in vitro activity against resistant isolates and sensitive control isolates [8].

The SENTRY Antimicrobial Surveillance Program has monitored linezolid before, during and after its release by various regulatory agencies. Among staphylococci, linezolid was active against all isolates (MICs, ≤ 4 g/l) regardless of susceptibility patterns of other antimicrobial agents. It was also consistently active against streptococci (MIC_{90}, 1 g/l). Linezolid remained active (MIC, ≤ 4 g/l) against all Gram-positive species strains tested in the SENTRY Program (1998–2000) [9]. However, sporadic in vitro resistance has been described in clinical isolates of MRSA in patients who have received linezolid therapy [10]. Point mutations in the gene encoding for the 23S bacterial ribosome appear to be the mechanism culminating in acquired resistance to linezolid in MRSA [11]. At the current time, this phenomenon remains extremely uncommon.

It must be highlighted that linezolid exerts post-antibiotic effects (PAEs). Mean maximal PAEs against strains of *S. aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterococcus faecium* and *S. pneumoniae* are 2.2, 1.8, 2.8, 2.0 and 3.0 h, respectively [12]. Resistance to methicillin or vancomycin (for the staphylococci), and penicillin (for the pneumococci) have no effect on the duration of the PAE.

2.2. Pharmacokinetics and pharmacodynamics

In general, linezolid produces a higher plasma concentration profile for a given dose in comparison to eperezolid. The mean C_{max} of linezolid (600 mg every 12 h for a total of five doses) in plasma was 18.3 g/l, with a T_{max} of 0.7 h after administration of the fifth oral dose (Table 2) [13]. The mean $t_{1/2}$ from plasma was 4.9 h (range, 2.9–7.9 h). The AUC_{last} and $AUC_{0-\infty}$ were 107.5 and 140.3 g h/l, respectively. Linezolid penetrated into the inflammatory fluid rather rapidly, the mean T_{max} being 3 h (range, 2–4 h). The mean C_{max} in the inflammatory fluid was 16.4 g/l (range, 6.8–36.8 g/l). The mean $t_{1/2}$ of linezolid from the inflammatory exudate was 5.7 h, slightly greater than that from plasma. The mean percentage of penetration of linezolid into inflammatory fluid was 104% (range, 80–130%). The degree of protein binding of an antimicrobial can play a major role in determining the level of tissue penetration [14]. The plasma protein binding of linezolid is relatively low, at 31% [15]. The degree of drug penetration into well-perfused areas of the body is also related to the volume of distribution. The steady-state V_d for linezolid has been shown to be approximately 50 l.

In healthy adult male subjects, after a treatment with linezolid (600 mg every 12 h for a total of five doses), concentrations in plasma, epithelial lining fluid (ELF), and alveolar cells (AC), respectively, were 7.3, 64.3, and 2.2 g/l at the 4-h bronchoalveolar lavage (BAL) time point and 7.6, 24.3, and 1.4 g/l at the 12-h BAL time point [16]. Linezolid concentrations in plasma, ELF, and AC declined monoexponentially, with half-lives of 6.9, 7.0, and 5.7 h, respectively. For a MIC of 4, the 12-h plasma AUC/MIC and maximum concentration/MIC ratios were 34.6 and 3.9, respectively, and the percentage of time the drug remained above the MIC for the 12-h dosing interval was 100%; the corresponding ratios in ELF were 120 and 16.1, respectively, and the percentage of time the drug remained above the MIC was 100%.

The area under the serum concentration-time curve to the MIC (AUC_{24}/MIC) ratios of linezolid for *S. aureus* and *S. pneumoniae* are 215 and 107.5, respectively. C_{max}/MIC ratio is 9.1. The time that concentrations of the drug in plasma remain above the MIC is at least 12 h, which is about 100% of the dosing interval when a twice-daily regimen is employed [13]. It has been demonstrated that the free-fraction pharmacodynamic parameters predictive of outcome in a rat model of pneumococcal pneumonia were a value of $>39\%$ for the percentage of time in the experimental dosing interval during which the linezolid concentration exceeded the MIC and a value of >147 for the ratio of the AUC_{24}/MIC [17].

2.3. Clinical and bacteriological experience

Linezolid was effective in patients with community-acquired pneumonia (CAP) requiring hospitalization, and significantly better when patients were bacteremic. In particular, empiric i.v./oral linezolid was more effective than ceftriaxone/cefpodoxime in patients hospitalized with CAP, with comparable cure rates in *S. pneumoniae* pneumonia and higher cure rates in pneumonia complicated by bacteremia [18] Linezolid has also been evaluated for the treatment of CAP in 66 hospitalized children. Sixty-one

Table 2
Pharmacokinetic parameters for linezolid in plasma and inflammatory fluid after five doses of 600-mg tablets at 12-h intervals. Adapted from Gee et al. [13]

Source of fluid	Mean ± SD (range [minimum – maximum])					
	C_{max} (g/l)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{last} (g h/l)	$AUC_{0-\infty}$ (g h/l)	% Penetration
Plasma	18.3 ± 6.0	0.7 ± 0.3	4.9 ± 1.8	107.5 ± 40.6	140.3 ± 73.1	
Blister	16.4 ± 10.6	3.0 ± 0.6	5.7 ± 1.7	101.6 ± 63.0	155.3 ± 80.1	104 ± 20.7

patients (92.4%) were considered cured including all the patients with proven pneumococcal pneumonia, one failed (MRSA) and 4 were considered indeterminate [19].

Hospitalized adults with known or suspected MRSA-induced pneumonia were treated with linezolid (600 mg twice daily) or vancomycin (1 g twice daily). At the test-of-cure visit, among evaluable patients with MRSA, there was no statistical difference between the two treatment groups with respect to clinical cure rates or microbiological success rates. Both regimens were well tolerated, with similar rates of adverse events [20].

A recent study evaluated the efficacy and safety of linezolid plus aztreonam compared with vancomycin plus aztreonam in the treatment of suspected nosocomial pneumonia due to a Gram-positive organism in adult patients [21]. The combination with linezolid demonstrated clinical cure and microbiological success rates equivalent to those reported with that including vancomycin in the intended to treat, clinically evaluable, and microbiologically evaluable populations (Fig. 1). Eradication rates of MRSA and safety evaluations were similar between treatment groups. As expected, patients in both treatment groups at risk for a concomitant or subsequent Gram-negative infection, including those who underwent intubation at baseline (about 57% of patients per treatment group) and/or who had high APACHE II scores, demonstrated lower cure rates.

2.4. Side effects

Data accrued from 1498 patients who received the drug in 5 randomized, comparative clinical trials have shown that the overall frequency of adverse events was 58.6% (878 of 1498) in the linezolid group and 52.4% (767 of 1464) in the comparator group [22]. Disturbances of gastrointestinal function, such as diarrhoea (8.3%), nausea (6.6%), and vomiting (4.3%), were the most commonly observed side effects. Less common adverse events included headache, taste alterations, vaginal moniliasis and other fungal infections, tongue discoloration, and abnormal findings on liver function tests. The incidence of these side effects was not significantly greater than in those patients treated with the standard comparator agent(s) nor were serious or irreversible sequelae observed. Discontinuation of linezolid because of the above symptoms occurred in fewer than 4% of cases.

Thrombocytopenia (below 75% of lower limit of normal and/or baseline platelet count), which occurred in 2.4% of patients in comparative trials and 2.6% of compassionate-use patients, was associated with linezolid therapy for >10 days [23,24]. Anaemia due to reversible red blood cell hypoplasia and bone marrow changes showing vacuolated erythroblasts in patients receiving longer courses of linezolid have been reported since linezolid was approved by the Food and Drug Administration (FDA) [22]. Therefore, even patients who are not considered to be at risk for development of thrombocytopenia or anaemia should be monitored closely if linezolid therapy is continued for >10 days [24].

2.5. Pharmacoeconomic studies

Oxazolidinones are very expensive. Therefore their use is justified only when they definitely offer an advantage over the cheaper vancomycin. No formal cost-efficacy analyses have been published, but the direct cost impact must be considered in light of the considerable aggregate morbidity and associated costs of serious Gram-positive infection and the cost of other efficacious antimicrobial

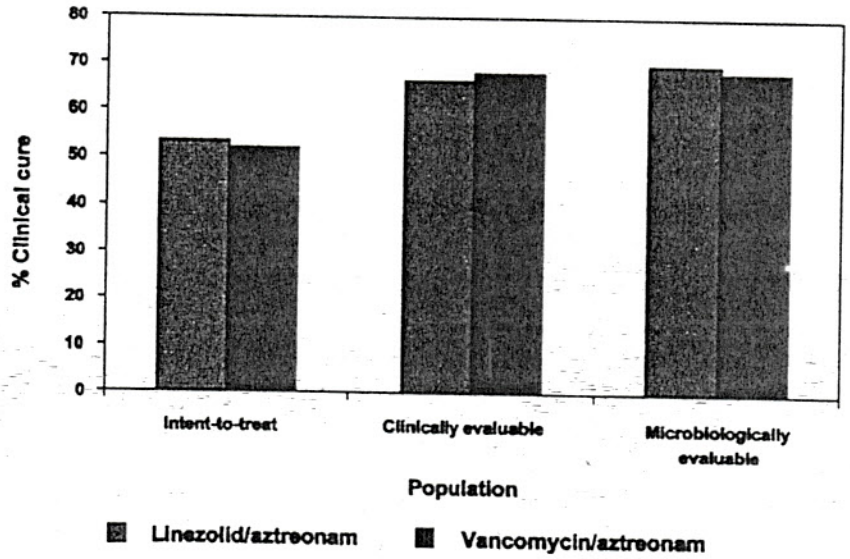

Fig. 1. Efficacy of linezolid plus aztreonam compared with vancomycin plus aztreonam in the treatment of suspected nosocomial pneumonia due to a Gram-positive organism in adult patients. Clinical cure was defined as resolution or improvement in baseline symptoms and radiograph, with no further requirement for antimicrobial therapy. Adapted from Rubinstein et al. [20].

options. A recent randomized multicenter trial comparing linezolid with vancomycin for the treatment of patients with known or suspected MRSA species infections showed that patients treated with linezolid had a moderately shorter median length of hospital stay (LOS) than that of the vancomycin group: 1 day in the total intent-to-treat sample or 2 days in the clinically evaluable sample (neither was statistically significant) [25]. It must be highlighted that LOS typically represents about 70–90% of the total cost of treating serious infections. However, examination of only primary LOS end points in the intent-to-treat sample does not necessarily reveal treatment effects in the most unbiased or precise manner. A further analysis of this trial has documented that when the antibiotic treatment started, 27.1% of patients treated with linezolid were in the intensive care unit or step-down unit compared with 20.5% treated with vancomycin [26]. The data showed the median LOS was 21 days for patients whose treatment began in the intensive care or step-down unit, whereas the median LOS was only 13 days for patients whose treatment began in the general ward. Such an imbalance might have biased the treatment effect on LOS. Moreover, the percentages of patients with seven or more concomitant medical conditions was 33% in the linezolid group but only 26% in the vancomycin group; linezolid-treated patients also had a greater average number of concomitant medical conditions. The median LOS was longer for those with (18 days) than for those without (13 days) seven or more comorbid conditions. The trial enrolled patients with several infections and from various geographic regions. Mixing patients with a variety of indications and from different geographic regions certainly increased the variation of the LOS and thus made it difficult to detect the treatment effect on LOS for the entire sample. Using more appropriate, but less commonly used, methods, such as the log-logistic model and multivariate survival analysis, it has been possible to reveal that across several measures, linezolid-treated patients had significant LOS reductions that otherwise would be masked. The average reduction in LOS associated with linezolid treatment, based on the log-logistic model after correction for covariate effects, was 18.1% or 2.53 days at the median. This treatment effect on LOS may be important for economic analysis. In any case, the higher cost of linezolid (versus vancomycin) may be partially or completely offset by shorter hospital stay, given the option to convert to oral linezolid when patients rapidly respond to parenteral treatment.

2.6. Compounds under investigation

Additional advances have recently been attained in the oxazolidinone entities. The oxazolidinones PNU-107922, PNU-140457, PNU-172576 and PNU-176798 were evaluated for their activity against Gram-positive bacteria including MRSA, VRE and PRSP, *Haemophilus influenzae* and *Moraxella catarrhalis* as well as aqueous solubility [27–29]. PNU-288034, a more recent compound, is approximately 1 $\log_2$ dilution more active than linezolid against staphylococci and streptococci [30].

AZD2563 is another new oxazolidinone that has targeted activity against Gram-positive bacteria. Its activity against 250 highly resistant pneumococci and 267 drug-susceptible isolates was determined. The AZD2563 MICs for 50 and 90% of the strains tested were 1 and 2 g/l and 0.5 and 1 g/l, respectively, for the two isolate groups [31]. Compared with linezolid, the distribution of MICs were usually shifted by one or two dilution steps to lower values for AZD2563 [32].

VRC-3125 and VRC-3783, novel 4′-substituted oxazolidinones, are highly active against *C. pneumoniae* (MICs, 0.25 and 2 g/l) and display good antibacterial activity against other key pathogens such as *S. pneumoniae* (MIC, 0.5 g/l) and *S. aureus* (MIC, 1 g/l) [33].

Oxazolidinone antibacterial agents, where the morpholino group of linezolid was replaced with an N-substituted piperidinyloxy moiety, were synthesized and shown to be active against a variety of resistant and susceptible Gram-positive organisms. The functionality attached to the piperidine nitrogen was extensively varied to determine the SAR for this series. One of the most potent compounds, 11, showed in vivo efficacy upon subcutaneous administration in a *S. aureus* Smith murine systemic infection [34]. A new pyridine-substituted oxazolidinone, DA-7867, showed 4- to 8-fold better antibacterial activity than linezolid against Gram-positive and Gram-negative pathogens including multi-drug-resistant bacteria and yielded mainly bacteriostatic activities [35].

3. Cationic peptides

Cationic antimicrobial peptides are produced by all organisms, from plants and insects to human beings, as a major part of their immediately effective, non-specific defenses against infections. In particular, cationic peptides, including the human β-defensins-1 (hBD-1) and-2 (hBD-2) and the cathelicidin LL-37/hCAP-18, are effector molecules of the innate immune system of the airways [36–40]. They contribute to innate immunity by direct microbiocidal activity. In addition, defensins and LL-37/hCAP-18 have multiple other functions, such as the activation of inflammatory cells and the regulation of adaptive immunity [41–43].

3.1. Antimicrobial activities

These peptides are termed antimicrobial because they have unusually broad spectra of activity, not only against Gram-negative and Gram-positive bacteria but also against antibiotic-resistant bacteria, fungi, viruses, and parasites. They can also act in synergy with host molecules, such as other cationic peptides and proteins,

lysozyme, and also conventional antibiotics, to kill microbes. Although the exact mechanism by which they kill bacteria is not clearly understood, it has been shown that peptide-lipid interactions leading to membrane permeation play a role in their activity. In particular, cationic antibacterial peptides appear to form channels in the bacterial cytoplasmic membrane; this leads to collapse of the transmembrane proton gradient, thereby affecting energy generation and transport processes [44]. It has been suggested that the major target for the cationic peptides could be the cytoplasmic membrane if the propensity to disrupt the membrane is very high, but also that for many peptides the lethal action would occur in the bacterial cytoplasm [45]. Recently, it has been proposed a mechanism for the interaction between these peptides and bacterial membranes similar to the 'carpet model', wherein the Lys residues interact with the anionic phospholipid head groups in the bacterial membrane surface and the hydrophobic core portion of the peptide is then able to interact with the lipid bilayer, causing disruption of the bacterial membrane [46]. Selectivity for bacterial cells compared to host cells appears to result from a variety of factors including the absence of cholesterol in bacterial membranes, the high content of anionic lipids at the surface of the bacterial cytoplasmic membrane and differences in electrochemical gradients across bacterial and mammalian cell membranes.

That these peptides vary with regard to their length, amino acid composition, and next positive charge, but act via a common mechanism, may imply that other linear antimicrobial peptides that share the same properties also operated by the same mechanism [47]. Differences in bactericidal activities against various strains are most likely due to variability in the components of the bacterial membranes and how these peptides interact with those membranes. For instance, the outer membrane surface of Gram-negative bacteria includes lipopolysaccharides (LPSs), whereas in Gram-positive bacteria there is no outer membrane and the cell wall includes the acidic polysaccharides, the teichoic acids [48].

Some peptides are produced in large quantities at sites of infection/inflammation, and their expression can be induced by bacterial products such as endotoxic LPS and proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α). These peptides can have a high affinity for bacterial products, such as LPS, allowing them to modulate the host response and reduce the inflammatory response in sepsis. More recently, they have been found to interact directly with host cells to modulate the inflammatory process and innate defenses (Fig. 2) [48].

3.2. Classification

Currently there is great interest in peptide antibiotics, some of which are derived from endogenous antimicrobial peptides of animals [49]. More than 500 such peptides have been discovered. Antimicrobial proteins and peptides are diverse in their structure and mechanism by which they kill infectious agents. Many antimicrobial peptides, however, have a secondary structure based on either α helices or β sheets. In addition, most antimicrobial peptides are cationic, although amino acid usage varies, including arginine, histidine, and lysine [50,51]. Thousands of such molecules have been synthesized but just a few are now entering clinical trials.

Cationic peptides can be classified into several groups on the basis of sequence similarities, secondary and tertiary structure, function and origin [52,53]. Insect cecropins and amphibian magainins are the prototypes of the most-studied group (α-helices), which consist of linear peptides that form α-helices devoid of cysteine residues. Antimicrobial proteins with a high content of one or two amino acids,

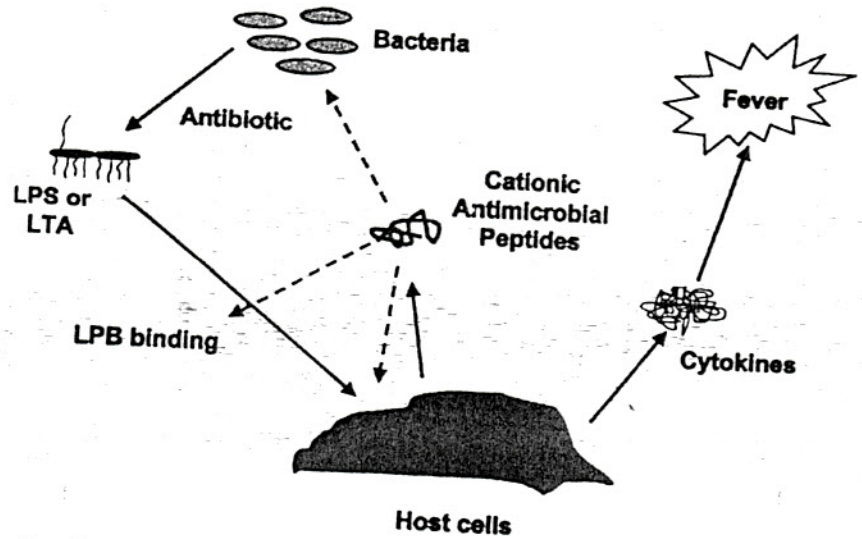

Fig. 2. Model outlining the major events in induction of sepsis by bacteria and the points at which cationic peptides are proposed to intervene.

particularly proline and glycine, such as insect drosocin and human histatin I, constitute the second class. The most diverse and widely distributed group is the cystine-rich peptides. This group (β-hairpins) includes the cysteine-stabilized αβ (CS αβ) motif of the insect defensins and the β-sheet structures of the porcine protegrins and amphibian tachyplesins. A fourth class of antimicrobial proteins recognized recently consists of macrocyclic peptides with tridisulfide structure, such as RTD-1 in primates, and macrocyclic peptides devoid of disulfides, such as AS-48 produced by *E. faecalis* and J25 from *E. coli*. Although the peptides in this fourth group share common structures and are positively charged, they vary considerably in chain length, hydrophobicity, and distribution of charges. The last group consists of peptides with unique structure, such as polymyxin B, which possesses a fatty acid attached through an amide linkage and a seven-member ring structure composed mainly of diaminobutyric acid.

3.3. Compounds in development that are potentially interesting for the treatment of LRTIs

Cationic peptides are in the focus of scientific research world-wide. In this field there are approximately 20 publications per month. The complexity of the situation would have deserved a complete review. Here we will describe some compounds that are potentially interesting for the treatment of LRTIs.

One prominent class of cationic antibacterial peptides comprises the α-helical class, which is unstructured in free solution but folds into an amphipathic α-helix upon insertion into the membranes of target cells. This class includes small peptides (typically 16–40 aa), e.g. magainins, cecropins, and dermaseptins, and larger proteins with multiple α helical domains, e.g. amoebapores, porcine NK-lysin, and human granulysin.

Magainins, cecropins, and dermaseptins are representatives of the amphipathic α-helical antimicrobial peptides. Recently, it has been documented that single-dose intraperitoneal magainins can result in significant bacterial growth inhibition, even though the strongest efficacy was reached only upon coadministration of the β-lactam piperacillin [54]. Furthermore, treatment with the magainins resulted in greater reductions in plasma endotoxin levels than treatment with piperacillin, confirming the double antimicrobial and antiendotoxin activities of the magainins. A synthetic derivative of magainin, pexiganan acetate (MSI-78) has shown broad-spectrum activity [55]. MICs for Gram-negative and Gram-positive bacteria, as well as for anaerobes, were 4–16 g/l (Table 3). Pexiganan acts with a bactericidal mechanism against which the likelihood of the development of resistance may be low. In addition, no evidence of cross-resistance to a number of other antibiotic classes has been observed, as determined by the equivalence of the MIC_{50}s and MIC_{90}s of pexiganan for those strains resistant to oxacillin, cefazolin, cefoxitin, imipenem, ofloxacin, ciprofloxacin, gentamicin, and clindamicin and the MIC_{50}s and MIC_{90}s of pexiganan for those strains susceptible to these antimicrobial agents [55].

Table 3
Distribution of MICs of pexiganan. Adapted from Ge et al. [55]

Microorganism	No. of isolates	MIC (g/l)	
		50%	90%
Gram-positive aerobes			
Staphylococcus aureus	512	8	16
Streptococcus pyogenes	253	4	8
Gram-negative aerobes			
Citrobacter freundii	105	8	16
Klebsiella pneumoniae	123	8	16
Pseudomonas aeruginosa	150	16	32
Gram-positive anaerobes			
Clostridium perfringens	31	16	64
Peptostreptococcus anaerobius	23	8	32
Gram-negative anaerobes			
Bacteroides fragilis	34	4	4
Fusobacterium nucleatum	29	8	64

Derivatives of the cytotoxic peptide dermaseptin S4, a 28-residues K4K20-S4 and two shorter versions, K4-S4(1-16) and K4-S4(1-13), have recently emerged as potential antimicrobial agents [56]. Compared with MSI-78, K4-S4(1-13) was at least as potent against bacteria (assessed at two MIC multiples) but displayed lesser toxicity against human erythrocytes. Serial passage in subinhibitory concentrations does not led to emergence of resistance to the L- or D isomer of either of the dermaseptin derivatives. In vivo bactericidal activity has been documented in neutropenic mice, where intraperitoneal administration of K4-S4(1-16) reduced the number of viable CFU in a dose-dependent manner by >3 log units within 1 h of exposure, and this was sustained for at least 5 h.

Also cathelicidin peptides adopt an α-helical structure, but only after binding to negatively charged bacterial cell wall components such as LPS or lipoteichoic acid [57]. In contrast to most conventional antibiotics, cathelicidin peptides have a rapid effect on bacteria. These changes presumably are associated with permeabilization of the bacterial cell membranes [58]. Because the bactericidal activity of peptides involves binding to cell wall components followed by disruption of bacterial membranes, it is unusual for bacteria to develop resistance to cathelicidins. In addition to their antimicrobial effects, there is evidence that the peptides' LPS binding activity reduces the incidence of septic shock in animal models of endotoxemia [59]. The bactericidal activities of five cathelicidin peptides (LL37 [human], CAP18 [rabbit], mCRAMP [mouse], rCRAMP [rat], and SMAP29 [sheep]), three novel α-helical peptides derived from SMAP29 and termed ovispirins (OV-1, OV-2, and OV-3), and two derivatives of CAP18 were tested by broth microdilution assays [60]. The sheep and rabbit cathelicidin-derived peptides exhibited potent activities against a large number of cystic fibrosis clinical isolates

of multiply antibiotic-resistant pathogens, including non-mucoid and mucoid strains of *P. aeruginosa* and some strains of *S. maltophilia*, and *A. xylosoxidans*. The 14- to 18-amino-acid ovispirins and the CAP18 derivatives also demonstrated significant activities.

Granulysin is a novel lytic molecule produced by human cytolytic T-lymphocytes (CTLs) and natural killer (NK) cells, which is highly homologous to NK-lysin, a cytotoxic and anti-microbial molecule expressed in porcine CTL and NK cells [61]. Its putative structure is a four α helical bundle similar to the amoebapore family members. Granulysin is active against a broad range of microbes, including Gram-positive and Gram-negative bacteria, parasites and *M. tuberculosis*. It is functionally related to other antibacterial peptides, like defensins and magainins, but is structurally distinct [62]. Peptides corresponding to the central region of granulysin lyse bacteria. Synthetic peptides derived from granulysin have differential activity against eukaryotic cells and bacteria. Compounds corresponding to either helix 2 or helix 3 lyse bacteria, while lysis of human cells and liposomes is dependent on the helix 3 sequence; the lysis of bacteria is unaffected by reduction of disulfide bonds [63] Initially a peptide (L-peptide) rich in five arginine residues and consisting of an 11-amino acid peptide (residues 32-42) of human granulysin was synthesized. Subsequently, the L-amino acids of the 11-amino acid peptide were replaced partially (D-peptide) or wholly (AD-peptide) with D-amino acids. Peptides with D-amino acid substitutions were found to lyse bacteria as efficiently as their all-L-amino acid parent, L-peptide. Since D-amino acid substitutions can make antimicrobial peptides resistant to proteolysis, this suggests that the antimicrobial peptides consisting of D-amino acids are potential candidates for clinical therapeutic use [64].

Histatins, which are naturally occurring peptides secreted by the salivary glands of humans, are known to have antibacterial properties [65]. Their derivatives could be effective agents for treatment of the chronic lung infections. Antimicrobial peptide P-113, a derivative of histatin 5, showed potent activity against the important respiratory pathogens, *P. aeruginosa*, *H. influenzae*, and *S. aureus* [66]. The results of animal studies [67] and phase I and II human clinical trials [68] indicate that the oral formulations have no adverse side effects. P-113 failed to retain its activity in sputum due to its instability, but a related derivative, P-113 with the amino acids in the D configuration (P-113D), retained good activity in presence of sputum [65]. It has been suggested that the use of P-113D would avoid selection of resistance to classical antibiotics, which might then be reserved for use in combating exacerbations [66].

Protegrins and their derivatives are a new class of peptide antibiotics based on mammalian antimicrobial peptides. Their pharmacological properties include an unusually broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi and some enveloped viruses. Protegrin-1 (PG-1), a 2 kDa cationic octadecapeptide (RGGRLCYCRRRFCVCVGR-amide) that is a member of the θ defensin-like family, was originally isolated from porcine leukocytes [69]. The antimicrobial spectrum of PG-1 includes *Chlamydia trachomatis*, *Candida albicans*, *E. coli*, *Fusobacterium nucleatum*, *Haemophilus ducreyi*, *Listeria monocytogenes*, *Neisseria gonorrhoeae*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *P. aeruginosa*, and *S. aureus*. It is rapidly bactericidal, functions well at elevated physiological salt concentrations such as those that may occur in the cystic fibrosis lung, and has a broad spectrum of activity. *P. aeruginosa* and *Burkholderia cepacia* infections of cystic fibrosis patients' lungs are often resistant to conventional antibiotic therapy [70–72]. *P. aeruginosa* strains examined were very sensitive to PG-1 (Fig. 3), exhibiting minimal active concentrations from 0.0625 to 0.5 g/l in radial diffusion assays. In contrast, all *B. cepacia* strains examined were greater than 10-fold to 100-fold more resistant, with minimal active concentrations ranging from 6 to 10 g/l. It has been suggested that the relative resistance of *B. cepacia* to protegrin is due to a reduced number of PG-1 binding sites on the lipid A moiety of its LPS [73].

The MICs of iseganan (IB-367), a synthetic protegrin analog, ranges from 0.13 to 64 g/l for Gram-positive bacteria (*Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus salivarius*, and *S. aureus*) and from 0.06 to 8 g/l for Gram-negative species (*Klebsiella*, *Escherichia*, and *Pseudomonas*). IB-367 exhibits rapid, microbicidal activity against both log- and stationary-phase cultures of MRSA and *P. aeruginosa* [74]. It has entered a phase I/II clinical trial as an aerosol formulation for respiratory infections in cystic fibrosis patients and as a gel formulation for the potential treatment of pneumonia [45,75].

The bactericidal/permeability-increasing protein (BPI) is a 55-kDa protein found in the primary (azurophilic) granules of human neutrophils and has also been detected on the neutrophil cell surface [76]. It is composed of two

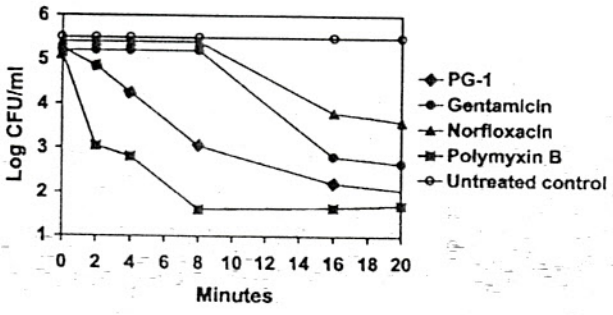

Fig. 3. Bactericidal activity of PG-1 against log-phase *P. aeruginosa*. Exponentially growing *P. aeruginosa* was treated with compounds at eight times the MICs. MICs (in micrograms per milliliter) were determined by the modified NCCLS method to be 0.5 for PG-1, 0.5 for gentamicin, 0.25 for norfloxacin, and 0.5 for polymyxin B. At the indicated times, survivors were enumerated by the pour plate method. The dashed line represents the minimum number of CFU that could be accurately determined. Adapted from Steinberg et al. [71].

similar domains, each containing a β-sheet and two α-helices. Two apolar pockets in BPI were discovered in the crystal structure. These pockets are located on the concave surface of the protein, each binding a molecule of phosphatidylcholine. It is hypothesized that these pockets may be sites of interaction between BPI and LPS or have some other important functional role. BPI does not manifest cytotoxicity against Gram-positive bacteria, fungi, or mammalian cells. BPI's cytotoxic activity is selectively manifest toward Gram-negative bacteria, including some encapsulated, serum-resistant clinical isolates such as *E. coli* K1/r [72]. This selectivity has been attributed to its high affinity (in the nanomolar range) for the lipid A moiety of LPS or endotoxin [77]. LPSs, which are the major components of the outer leaflet of the Gram-negative bacterial outer membrane, are stabilized by a regular array of divalent ions that serve to cross-link the negatively charged LPS molecules. Binding of BPI to Gram-negative bacteria competitively displaces these outer membrane calcium and magnesium ions [78]. BPI is also effective in vitro against *C. pneumoniae* [45]. A recombinant amino terminal fragment of BPI, $rBPI_{21}$ has antiendotoxic and antibacterial activities that can be demonstrated in biologic fluids and animal models. Human clinical trials have indicated that $rBPI_{21}$ is safe, without significant immunogenicity or toxicity [79]. $rBPI_{21}$ given intravenously to subjects who have received endotoxin is able to markedly inhibit LPS-induced cytokine release [80]. Polymyxin B-resistant *Acinetobacter baumannii* clinical isolate have been shown to be susceptible to $rBPI_{21}$ [81].

Mayo et al. [82] have designed a series of synthetic peptide 33mers (βpep peptides) by incorporating β-conformation stabilizing residues from the β-sheet domains of CXC-Chemokines and functionally important residues from the β-sheet domain of the human neutrophil bactericidal protein BPI. These peptides retain the bactericidal activity of the BPI.

4. Lipopeptides

Daptomycin, a unique acidic lipopeptide antibiotic (Fig. 4) not really new, since it was discovered in the early 1980s, but abandoned at that time because of an unfavorable side effect profile at the dosing given then, is an antimicrobial agent with bactericidal activity against all clinically important Gram-positive bacteria, including resistant pathogens such as vancomycin-resistant enterococci (VRE), MRSA, glycopeptide intermediately susceptible *S. aureus* (GISA), coagulase-negative staphylococci, and DRSP [83,84]. It is a fermentation product of *Streptomyces roseosporus*.

Daptomycin kills bacteria by disrupting multiple aspects of bacterial plasma membrane function, included inhibition of peptidoglycan synthesis, inhibition of lipoteichoic acid synthesis and dissipation of bacterial membrane potential, while not penetrating into the cytoplasm [85,86]. It acts by specifically inhibiting the synthesis of the cell wall, and in *S. aureus* it inhibits the incorporation of ^{14}C]alanine into peptidoglycan.

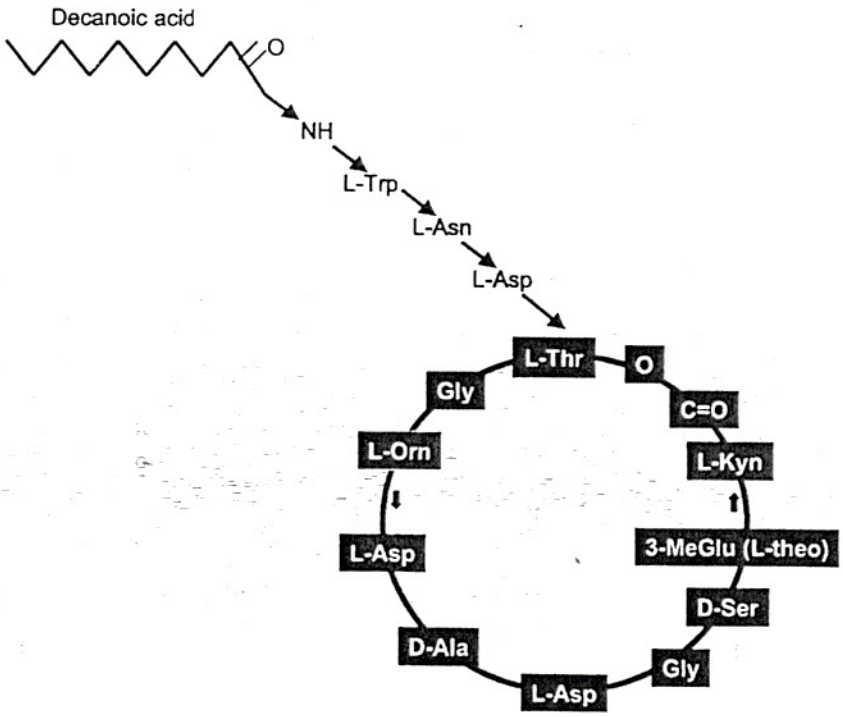

Fig. 4. Amino acid structure and location of decanoic acid side chain of daptomycin.

4.1. Antibacterial spectrum

The MIC value for daptomycin against susceptible strains is typically 4 times less than that of vancomycin [78], although against methicillin-susceptible *S. aureus* and MRSA, daptomycin activity can also be approximately 8- to 30-fold greater than those of vancomycin, linezolid and quinupristin–dalfopristin [87]. The MICs at which 90% of isolates are inhibited for daptomycin and vancomycin, respectively, were as follows: MRSA, 1 and 2 g/l; MRSS, 1 and 4 g/l; and PRSP, 1 and 0.5 g/l (Table 4) [88]. Daptomycin was active against all strains of a spectrum of pneumococci with varying susceptibilities to β-lactams, macrolides and quinolones (MIC range ≤0.5 mg/l; MIC_{50}, 0.125 mg/l; MIC_{90}, 0.25 mg/l). All pneumococci were susceptible to vancomycin, teicoplanin, linezolid and quinupristin/dalfopristin, with MICs <4.0 mg/l [89].

It has been showed that the addition of calcium ions to a final concentration of 50 mg/l enhanced the activity of daptomycin generally by 8- to 16-fold. In the presence of calcium ions daptomycin was uniformly active against the strains of *Staphylococcus* spp. and *Streptococcus* spp. studied with a MIC_{90} of ≤1 mg/l [90]. Vancomycin-, fluoroquinolone- and quinupristin/dalfopristin-resistant strains were all susceptible to daptomycin. The presence of serum reduced the apparent activity of daptomycin to only a moderate extent [90]. Antibacterial activity against resistant strains is comparable to that against susceptible strains [84]. Development of resistance is unlikely when therapeutic serum levels of daptomycin are maintained [91].

Daptomycin possesses a concentration-dependent killing and an extended PAE of 3–6 h, which lends itself to once-daily administration [92].

4.2. Pharmacokinetics and pharmacodynamics

With once-daily administration, daptomycin exhibits linear pharmacokinetics and minimal accumulation with doses up to 6 mg/kg in healthy volunteers [83]. Plasma clearance is low, resulting in part from high protein binding (87–94%) [93]. Excretion of the drug occurs primarily via the kidney, with approximately 80% of the total dose, of which two-thirds is intact drug, recovered in the urine. Daptomycin has been administered intravenously at a dose of 4 mg/kg of body weight to seven healthy male volunteers [94]. The mean peak concentrations in plasma and inflammatory fluid were 77.5 and 27.6 g/l, respectively; the mean terminal elimination half-lives were 7.74 and 13.2 h, respectively. The overall penetration of total drug into the inflammatory fluid (measured by ratio of the area under the concentration-time curve from 0 to 24 h for inflammatory fluid compared with that for plasma) was 68.4%. The mean urinary recovery over 24 h was 59.7%.

Woodworth et al. [95] reported that a 6-mg/kg dose of daptomycin results in a 24-h AUC of 598 ± 110 g h/l in healthy human volunteers, but with individual variations. This finding is important because in a murine neutropenic thigh model of *S. aureus* infection daptomycin presents a concentration-dependent bactericidal activity and it has been observed that the AUC/MIC ratio is the dynamically linked variable, considering that [63]. This experimental study has demonstrated that for an *S. aureus* isolate for which the MIC is 0.2 g/l, the mean stasis dose and ED_{80} of 6.35 and 13.55 mg of daptomycin/kg are associated with AUCs of 49.1 and 103.3 g h/l, respectively. Thus, the AUC/MIC ratios for the stasis and 80% maximal kill targets are 245.5 and 516.5, respectively. These target AUC/MIC ratios should be readily exceeded in most patients treated with 6 mg of daptomycin/kg. Higher doses of daptomycin may be required to eradicate infections due to *S. aureus* isolates for which MICs are greater than 1 g/l, the MIC_{90} for daptomycin. A clinical dose of 6 mg of daptomycin/kg should result in an AUC that readily exceeds 245.5 g·h/l, the stasis AUC for bacterial isolates for which MICs are up to 1 g/l. Based on the individual variation in AUCs for daptomycin reported by Woodworth et al. [95], it has been suggested that the 80% maximal kill target of 516 g h/l may not be achieved in 40–45% of patients with *S. aureus* infections due to isolates with MICs of 1 g/l. Since the daptomycin MIC for less than 10% of *S. aureus* isolates is >1 g/l, it has been predicted that approximately 80% of all patients with *S. aureus* infections will be responsive to a 6-mg/kg dose, while 20% may require adjustment of the

Table 4
Susceptibilities of Gram-positive isolates to daptomycin and vancomycin. Adapted from Snydman et al. [88]

Microorganism	No. of isolates	MIC (g/l)			
		Daptomycin		Vancomycin	
		50%	90%	50%	90%
MRSA	54	1	1	2	2
MSSA	27	0.25	1	0.5	1
S. pneumoniae (penicillin susceptible)	16	0.03	0.125	0.0125	0.25
S. pneumoniae (penicillin intermediate)	21	0.25	1	0.25	0.5
S. pneumoniae (penicillin resistant)	24	0.5	1	0.25	0.5
S. pyogenes	10	0.015	0.125	0.25	0.5

total daily daptomycin dose to ensure that target AUCs are achieved for optimal therapeutic outcome [96].

Daptomycin clearance is highly correlated with creatinine clearance. Pharmacokinetic parameters C_{max}, V_d, $CL_{nonrenal}$ were not statistically different between subjects with graded renal function and end-stage renal disease who received 4 mg/kg of daptomycin intravenously over 30 min, with the exception of C_{max} (decreased) and V_d (increased) for subjects with end-stage renal disease on hemodialysis. Clinically significant changes in daptomycin CL_p and $T_{1/2}$ did not occur until severe renal disease or end-stage renal disease. No alteration in the daptomycin dose or dosage regimen should be required in patients whose creatinine clearance is >30 ml/min. Dose or dosage regimen adjustment will be necessary in patients with severe renal disease or end-stage renal disease [97].

4.3. Clinical and bacteriological experience

Clinical trials conducted in the early 1990s demonstrated that daptomycin was equivalent to conventional therapy in the treatment of skin and soft tissue infections and bacteremia due to Gram-positive bacteria. The failure rate for daptomycin in the treatment of *S. aureus* endocarditis was higher than expected [83]. However, the latter trial did not evaluate the full potential of daptomycin, since complete dose-range studies and pharmacodynamic studies were not available when the clinically studied dosage was selected. With the increasing incidence of infections due to MRSA, PRSP, and other multiantibiotic-resistant bacteria, there is a renewed interest in evaluating the activity of daptomycin for he treatment of infections due to Gram-positive bacteria.

Unfortunately, although the pharmacologic profile of laptomycin is interesting and indicates a potential role in ne treatment of community- or nosocomial-acquired TRIs, studies indicating its efficacy in these pathologies re still lacking in the literature.

4. Side effects

Only at the highest multiple-dose level evaluated were ptomycin found to be associated with mild, reversible eletal muscle adverse events with a myopathy that is aracterized by minimal degeneration, with regeneration in absence of fibrosis [84]. Extrapolation of the results of estigative studies with dogs to humans suggests that e-daily dosing should result in a lower incidence of letal-muscle effects in patients than the same total daily e administered on a fractionated regimen [98].

In two randomized, evaluator-blinded, phase III studies h a combined enrolment of 1079 subjects suffering from plicated skin and soft tissue infections overall adverse nt patterns associated with DAP and standard therapy i-staphylococcal penicillin 4–12 g/day or vancomycin q12 h) were similar; nausea (5.4%, 9.0%), constipation (5.4%, 6.5%) and headache (5.2%, 5.0%) were the events most reported, respectively [99].

Although daptomycin may displace other protein-bound drugs, there is low potential for interference with hepatically metabolized drugs [83].

5. Peptide deformylase inhibitors

Peptide deformylase (PDF) is a prokaryotic metalloenzyme that is essential for bacterial growth and is a new target for the development of antibacterial agents. The nascent synthesized polypeptide is converted to mature protein through sequential removal of *N*-formyl group and methionine by PDF and methionine aminopeptidase. The enzyme is lacking in mammalian cells and could be a target for antibacterial agents. Recently, a number of inhibitors of PDF have been described to have antibacterial activity.

Actinonin, a naturally occurring antibacterial agent, is a potent PDF inhibitor. Microbiological evaluation revealed that it is a bacteriostatic agent with activity against Gram-positive and fastidious Gram-negative microorganisms, but the level of activity is low (MIC, >2 g/l) for *S. epidermidis*. MICs of >8 g/l were found against other Gram-positive cocci; against fastidious Gram-negative bacteria, MICs were 0.25–4 g/l [100]. Early attempts to develop a series of hydroxamic acid analogues of actinonin never reached clinical development due to poor in vivo activity [101]. BB-3497, an *N*-formyl-hydroxylamine derivative, is a potent PDF inhibitor with good selectivity for mammalian metalloenzymes and activity against Gram-negative and Gram-positive pathogens, including multidrug-resistant strains. It is well absorbed following p.o. administration and is effective in animal models of infection, validating the potential of PDF as an antibacterial target [102].

A new class of PDF inhibitors with *N*-alkyl urea at the P_1' site has been identified. Compounds with MICs of ≤4 g/l against Gram-positive and Gram-negative pathogens, including *S. aureus*, *S. pneumoniae*, and *H. influenzae*, have been identified. The concentrations needed to inhibit 50% of enzyme activity (IC_{50}s) for *E. coli* Ni-PDF were ≤0.1 μM, demonstrating the specificity of the inhibitors. In addition, these compounds were very selective for PDF, with IC_{50}s of consistently >200 μM for matrilysin and other mammalian metalloproteases [103].

Recently, it has been shown that the potency of NVP-PDF386 (VRC4887), another new peptide deformylase inhibitor, compared favorably with those of control compounds, including glycopeptides, oxazolidinones, a streptogramin combination and other agents with activity focused against Gram-positive cocci [104].

Modification of the P3′ dimethylamide substituent provided a novel series of PDF inhibitors, with improved activity against a range of pathogenic organisms. Compounds from this series, as exemplified by BB-83698, show good in vitro activity against the major Gram-positive

respiratory pathogens, including quinolone- and penicillin-resistant pneumococci, and MRSA. The potent in vitro activity of BB-83698 against *S. pneumoniae*, translates into good in vivo efficacy in a mouse model of pneumococcal pneumonia. BB-83698, administered orally at 80 mg/kg twice daily for three days resulted in survival of 90–100% of animals 10 days post infection, irrespective of the penicillin, macrolide or fluoroquinolone resistance of the infecting strain [105].

The microbiologic profile and resistance frequency suggest these agents will be most appropriate for respiratory infections as a first target. In any case, even though potent inhibitors of PDF have been discovered, their bacteriostatic mechanism of action and the rapid development of resistance in vitro may limit their potential as antibacterial drugs.

6. Pleuromutilins

Pleuromutilins are natural compounds that are not currently used in human medicine but instead in veterinary medicine. These drugs are strong inhibitors of peptidyl transferase and interact with domain V of 23S RNA. Pleuromutilin also acts as the building block for the production of tiamulin.

Beginning with pleuromutilin, structure–activity studies yielded two semisynthetic derivatives, SB-247386 and SB-268091, which were selected for further investigation. In vitro, SB-268091 is particularly active against methicillin-susceptible *S. aureus* (MIC_{90}, 0.06 g/l), methicillin-resistant (MIC_{90}, 0.06 g/l), strains, as well as against *S. epidermidis* (MIC_{90}, 0.03 g/l) or *S. pyogenes* (MIC_{90}, $\leq$0.01 g/l). SB-247386 is less active than SB-268091 [106]. A novel series of mutilin 14-carbamates has also been discovered. In particular, the 4-methoxybenzoylcarbamate, SB-222734 displays potent antibacterial activity against a number of bacterial pathogens which are resistant to currently used agents and shows enhanced metabolic stability when compared to earlier pleuromutilin derivatives. Such derivatives therefore have the potential to provide a new class of antibacterial agents for human therapy which address the threat of bacterial resistance [107].

It must been highlight that, although valnemulin is active effective in the treatment of enzootic pneumonia and acute polyarthritis in pigs, it has also been used for treating resistant mycoplasma infection in immunocompromised patients. Valnemulin completely eradicated the infection in two patients, with the emergence of a resistant strain in the third [108].

7. Conclusion

The possibility of having structurally novel drugs is extremely exiting. In fact, it allows to effectively fight against bacteria that now are scarcely controlled by the traditional antimicrobial agents [109]. The true problem is to convince physicians to reserve these antibiotics only to cases of documented ineffectiveness of the common antimicrobial agents. This conservative approach, that is also justified by the high cost of novel antibiotics, contrasts with the physicians' fear of prescribing an ineffective treatment to patients who are often in severe clinical condition, and, more important, with the pressure of Drug Companies that invest a large quantity of money for developing new compounds and need return charges. The best way for utilizing all more recent antimicrobial agents is to produce balanced guidelines that clearly indicate when the prescription of these agents can be considered and, consequently, the National Health Systems or the insurance companies must be responsible for their cost.

References

[1] Cazzola M, Blasi F, Centanni S, et al. Advances in the research and development of chemotherapeutic agents for respiratory tract bacterial infections. Pulm Pharmacol Ther 2001;14:367–81.
[2] Slee AM, Wuonola MA, McRipley RJ, et al. Oxazolidinones, a new class of synthetic antibacterial agents: in vitro and in vivo activities of DuP 105 and DuP 721. Antimicrob Agents Chemother 1987;31: 1791–7.
[3] Shinabarger DL, Marotti KR, Murray RW, et al. Mechanism of action of oxazolidinones: effects of linezolid and eperezolid on translation reactions. Antimicrob Agents Chemother 1997;41: 2132–6.
[4] Swaney SM, Aoki H, Ganoza MC, et al. The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. Antimicrob Agents Chemother 1998;42:3251–5.
[5] Dresser LD, Rybak MJ. The pharmacologic and bacteriologic properties of oxazolidinones, a new class of synthetic antimicrobials. Pharmacotherapy 1998;18:456–62.
[6] Pawsey SD, Daley-Yates PT, Wajszczuk CP, et al. U-100766 safety, toleration and pharmacokinetics after oral and intravenous administration. Program and abstracts of the First European Congress of Chemotherapy; 1996. p. F151.
[7] Cercenado E, García-Garrote F, Bouza E. In vitro activity of linezolid against multiply resistant Gram-positive clinical isolates. J Antimicrob Chemother 2001;47:77–81.
[8] Fines M, Leclercq R. Activity of linezolid against Gram-positive cocci possessing genes conferring resistance to protein synthesis inhibitors. J Antimicrob Chemother 2000;45:797–802.
[9] Mutnick AH, Biedenbach DJ, Turnidge JD, et al. Spectrum and potency evaluation of a new oxazolidinone, linezolid: report from the SENTRY Antimicrobial Surveillance Program, 1998–2000. Diagn Microbiol Infect Dis 2002;43:65–73.
[10] Tsiodras S, Gott HS, Sakoulas G, et al. Linezo-lid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet 2001;358: 207–8.
[11] Prystowsky J, Siddiqui F, Chosay J, et al. Resistance to linezolid: characterization of mutations in rRNA and comparison of their occurrences in vancomycin-resistant enterococci. Antimicrob Agents Chemother 2001;45:2154–6.
[12] Munckhof WJ, Giles C, Turnidge JD. Post-antibiotic growth suppression of linezolid against Gram-positive bacteria. J Antimicrob Chemother 2001;47:879–83.
[13] Gee T, Ellis R, Marshall G, et al. Pharmacokinetics and tissue penetration of linezolid following multiple oral doses. Antimicrob Agents Chemother 2001;45:1843–6.

[14] Hyatt JM, McKinnon PS, Zimmer GS, et al. The importance of pharmacokinetic/pharmacodynamic surrogate markers of outcome. Focus on antibacterial agents. Clin Pharmacokinet 1995;28:143–60.
[15] Ford C, Hamel J, Stapert D, et al. Oxazolidinones: a new class of antimicrobials. Infect Med 1999;16:435–45.
[16] Conte Jr JE, Golden JA, Kipps J, et al. Intrapulmonary pharmacokinetics of linezolid. Antimicrob Agents Chemother 2002;46: 1475–80.
[17] Gentry-Nielsen MJ, Olsen KM, Preheim LC. Pharmacodynamic activity and efficacy of linezolid in a rat model of pneumococcal pneumonia. Antimicrob Agents Chemother 2002;46:1345–51.
[18] San Pedro GS, Cammarata SK, Oliphant TH, et al. Linezolid versus ceftriaxone/cefpodoxime in patients hospitalized for the treatment of Streptococcus pneumoniae pneumonia. Scand J Infect Dis 2002;34: 720–8.
[19] Kaplan SL, Patterson L, Edwards KM, et al. Linezolid for the treatment of community-acquired pneumonia in hospitalized children. Linezolid Pediatric Pneumonia Study Group. Pediatr Infect Dis J 2001;20:488–94.
[20] Rubinstein E, Cammarata S, Oliphant T, et al. Linezolid (PNU-100766) versus vancomycin in the treatment of hospitalized patients with nosocomial pneumonia: a randomized, double-blind, multicenter study. Clin Infect Dis 2001;32:402–12.
[21] Stevens DL, Herr D, Lampiris H, et al. Linezolid versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Clin Infect Dis 2002;34:1481–90.
[22] Linden P. Use of linezolid for Gram-positive infections. Infect Med 2002;19:25–32.
[23] Green SL, Maddox JC, Huttenback ED. Linezolid and reversible myelosuppression. JAMA 2001;285:1291.
[24] Attassi K, Hershberger E, Alam R, et al. Thrombocytopenia associated with linezolid therapy. Clin Infect Dis 2002;34:695–8.
[25] Li Z, Willke RJ, Pinto LA, et al. Comparison of length of hospital stay for patients with known or suspected methicillin-resistant *Staphylococcus* species infections treated with linezolid or vancomycin: a randomized, multicenter trial. Pharmacotherapy 2001;21: 263–74.
[26] Li JZ, Willke RJ, Rittenhouse BE, et al. Approaches to analysis of length of hospital stay related to antibiotic therapy in a randomized clinical trial: linezolid versus vancomycin for treatment of known or suspected methicillin-resistant *Staphylococcus* species infections. Pharmacotherapy 2002;22:45S–54S.
[27] Genin MJ, Hutchinson DK, Allwine DA, et al. Nitrogen-carbon-linked (azolylphenyl)oxazolidinones with potent antibacterial activity against the fastidious Gram-negative organisms *Haemophilus influenzae* and *Moraxella catarrhalis*. J Med Chem 1998;41:5144–7.
[28] Barbachyn MR, Brickner SJ, Gadwood RC, et al. Design, synthesis, and evaluation of novel oxazolidinone antibacterial agents active against multidrug-resistant bacteria. Adv Exp Med Biol 1998;456: 219–38.
[29] Genin MJ, Allwine DA, Anderson DJ, et al. Substituent effects on the antibacterial activity of nitrogen-carbon-linked (azolylphenyl)oxazolidinones with expanded activity against the fastidious Gram-negative organisms *Haemophilus influenzae* and *Moraxella catarrhalis*. J Med Chem 2000;43:953–70.
[30] Brown SD, Barry AL, Fuchs PC, et al. PNU-288034: in vitro potency compared to linezolid, spectrum of antimicrobial activity, and confirmation of disk mass. Program and abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, California; September 27–30, 2002. Abst F-1337
[31] Baum SE, Crawford SA, McElmeel ML, et al. Antimicrob Agents Chemother 2002;46:3094–5.
[32] Fluit AC, Schmitz FJ, Verhoef J, et al. In vitro activity of AZD2563, a novel oxazolidinone, against European Gram-positive cocci. J Antimicrob Chemother 2002;50:271–6.
[33] Kubo A, Press B, Luehr GW, et al. In vitro activity of novel oxazolidinones against *Chlamydia*. Program and abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, California; September 27–30, 2002. Abst F-1322
[34] Weidner-Wells MA, Boggs CM, Foleno BD, et al. Novel piperidinyloxy oxazolidinone antibacterial agents. Diversification of the N-substituent. Bioorg Med Chem 2002;10:2345–51.
[35] Choi S, Lee T, Im W, et al. In vitro antibacterial activity of DA-7867, a new oxazolidinone. Program and abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, California; September 27–30, 2002. Abst F-1313
[36] Ganz T. Defensins and host defense. Science 1999;286:420–1.
[37] Goldman MJ, Anderson GM, Stolzenberg ED, et al. Human β-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell 1997;88:553–60.
[38] McCray Jr P, Bentley L. Human airway epithelia express a β-defensin. Am J Respir Cell Mol Biol 1997;16:343–9.
[39] Harder J, Bartels J, Christophers E, et al. A peptide antibiotic from human skin. Nature 1997;387:861.
[40] Bals R, Wang X, Zasloff M, et al. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. Proc Natl Acad Sci USA 1998;95:9541–6.
[41] Yang D, Chen Q, Schmidt AP, et al. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T-cells. J Exp Med 2000;192:1069–74.
[42] Yang D, Chertov O, Bykovskaia S, et al. β-Defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 1999;286:525–8.
[43] Le Y, Li B, Gong W, et al. Novel pathophysiological role of classical chemotactic peptide receptors and their communications with chemokine receptors. Immunol Rev 2000;177:185–94.
[44] Johansen C, Verheul A, Gram L, et al. Protamine-induced permeabilization of cell envelopes of Gram-positive and Gram-negative bacteria. Appl Environ Microbiol 1997;63:1155–9.
[45] Hancock REW, Nair P. Cationic antimicrobial peptide antibiotics. Curr Opin Anti-infect Invest Drugs 2000;2:140–4.
[46] Stark M, Liu LP, Deber CM. Cationic hydrophobic peptides with antimicrobial activity. Antimicrob Agents Chemother 2002;46: 3585–90.
[47] Oren Z, Shai Y. Mode of action of linear amphipathic α-helical antimicrobial peptides. Biopolymers 1998;47:451–63.
[48] Scott MG, Hancock RE. Cationic antimicrobial peptides and their multifunctional role in the immune system. Crit Rev Immunol 2000; 20:407–31.
[49] Hancock RE, Chapple DS. Peptide antibiotics. Antimicrob Agents Chemother 1999;43:1317–23.
[50] Andra J, Leippe M. Pore-forming peptide of *Entamoeba histolytica*. Significance of positively charged amino acid residues for its mode of action. FEBS Lett 1994;354:97–102.
[51] Cabiaux V, Agerberth B, Johansson J, et al. Secondary structure and membrane interaction of PR-39, a Pro rich antibacterial peptide. Eur J Biochem 1994;224:1019–27.
[52] Andreu D, Rivas L. Animal antimicrobial peptides: an overview. Biopolymers 1998;47:415–33.
[53] Ganz T, Lehrer RI. Antibiotic peptides from higher eukaryotes: biology and applications. Mol Med Today 1999;5:292–7.
[54] Cirioni O, Giacometti A, Ghiselli R, et al. Single-dose intraperitoneal magainins improve survival in a Gram-negative-pathogen septic shock rat model. Antimicrob Agents Chemother 2002;46:101–4.
[55] Ge Y, MacDonald DL, Holroyd KJ, et al. In vitro antibacterial properties of pexiganan, an analog of magainin. Antimicrob Agents Chemother 1999;43:782–8.
[56] Navon-Venezia S, Feder R, Gaidukov L, et al. Antibacterial properties of dermaseptin S4 derivatives with in vivo activity. Antimicrob Agents Chemother 2002;46:689–94.

[57] Travis SM, Anderson N, Forsyth WR, et al. Bactericidal activity of mammalian cathelicidin peptides. Infect Immun 2000;68:2748–55.
[58] Skerlavaj B, Benincasa M, Risso A, et al. SMAP29: a potent antibacterial and antifungal peptide from sheep leukocytes. FEBS Lett 1999;463:56–8.
[59] Hancock REW. Peptide antibiotics. Lancet 1997;349:418–22.
[60] Saiman L, Tabibi S, Starner TD, et al. Cathelicidin peptides inhibit multiply antibiotic-resistant pathogens from patients with cystic fibrosis. Antimicrob Agents Chemother 2001;45:2838–44.
[61] Andersson M, Gunne H, Agerberth B, et al. NK-lysin, a novel effector peptide of cytotoxic T and NK cells: structure and cDNA cloning of the porcine form, induction by interleukin 2, antibacterial and antitumour activity. EMBO J 1995;14:1615–25.
[62] Krensky AM, Okada S, Clayberger C, et al. Granulysin: a novel antimicrobial. Exp Opin Investigat Drugs 2001;10:321–9.
[63] Wang Z, Choice E, Kaspar A, et al. Bactericidal and tumoricidal activities of synthetic peptides derived from granulysin. J Immunol 2000;165:1486–90.
[64] Hamamoto K, Kida Y, Zhang Y, et al. Antimicrobial activity and stability to proteolysis of small linear cationic peptides with D-amino acid substitutions. Microbiol Immunol 2002;46:741–9.
[65] MacKay BJ, Denepitiya L, Iacono VJ, et al. Growth-inhibitory and bactericidal effects of human parotid salivary histidine-rich polypeptides on *Streptococcus mutans*. Infect Immun 1984;44:695–701.
[66] Sajjan US, Tran LT, Sole N, et al. P-113D, an antimicrobial peptide active against *Pseudomonas aeruginosa*, retains activity in the presence of sputum from cystic fibrosis patients. Antimicrob Agents Chemother 2001;45:3437–44.
[67] Paquette DW, Waters GS, Stefanidou VL, et al. Inhibition of experimental gingivitis in beagle dogs with topical salivary histatins. J Clin Periodontol 1997;24:216–22.
[68] Mickels N, McManus C, Massaro J, et al. Clinical and microbial evaluation of a histatin containing mouthrinse in humans with experimental gingivitis. J Clin Periodontol 2001;28:404–10.
[69] Kokryakov VN, Harwig SSL, Panyutich EA, et al. Protegrins: leukocyte antimicrobial peptides that combine features of corticostatic defensins and tachyplesins. FEBS Lett 1993;327:231–6.
[70] Harwig SSL, Waring AJ, Yang HJ, et al. Intramolecular disulfide bonds enhance the antimicrobial and lytic activities of protegrins at physiological sodium chloride concentrations. Eur J Biochem 1996; 240:352–7.
[71] Steinberg DA, Hurst MA, Fujii CA, et al. Protegrin-1: a broad-spectrum, rapidly microbicidal peptide with in vivo activity. Antimicrob Agents Chemother 1997;41:1738–42.
[72] Hancock REW, Lehrer RI. Cationic peptides a new source of antibiotics. Trends Biotech 1998;16:82–8.
[73] Albrecht MT, Wang W, Shamova O, et al. Binding of protegrin-1 to *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Respir Res 2002;14(3(1)):18.
[74] Mosca DA, Hurst MA, So W, et al. IB-367, a protegrin peptide with in vitro and in vivo activities against the microflora associated with oral mucositis. Antimicrob Agents Chemother 2000;44:1803–8.
[75] Toney JH. Iseganan (IntraBiotics pharmaceuticals). Curr Opin Investigat Drugs 2002;3:225–8.
[76] Levy O. A neutrophil-derived anti-infective molecule: bactericidal/permeability-increasing protein. Antimicrob Agents Chemother 2000;44:2925–31.
[77] Gazzano-Santoro H, Parent JB, Grinna L, et al. High-affinity binding of the bactericidal/permeability-increasing protein and a recombinant amino-terminal fragment to the lipid A region of lipopolysaccharide. Infect Immun 1992;60:4754–61.
[78] Mannion BA, Kalatzis ES, Weiss J, et al. Preferential binding of the neutrophil cytoplasmic granule-derived bactericidal/permeability increasing protein to target bacteria. Implications and use as a means of purification. J Immunol 1989;142:2807–12.
[79] Giroir BP, Quint PA, Barton P, et al. Preliminary evaluation of recombinant amino-terminal fragment of human bactericidal/permeability-increasing protein in children with severe meningococcal sepsis. Lancet 1997;350:1439–43.
[80] von der Mohlen MA, Kimmings AN, Wedel NI, et al. Inhibition of endotoxin-induced cytokine release and neutrophil activation in humans by use of recombinant bactericidal/permeability-increasing protein. J Infect Dis 1995;172:144–51.
[81] Urban C, Mariano N, Rahal JJ, et al. Polymyxin B-resistant *Acinetobacter baumannii* clinical isolate susceptible to recombinant BPI21 and Cecropin P1. Antimicrob Agents Chemother 2001;45: 994–5.
[82] Mayo KH, Haseman J, Ilyina E, et al. Designed β-sheet-forming peptide 33mers with potent human bactericidal/permeability increasing protein-like bactericidal and endotoxin neutralizing activities. Biochim Biophys Acta 1998;1425:81–92.
[83] Tally FP, Zeckel M, Wasilewski MM, et al. Daptomycin: a novel agent for Gram-positive infections. Exp Opin Invest Drugs 1999;8: 1223–38.
[84] Tally FP, DeBruin MF. Development of daptomycin for Gram-positive infections. J Antimicrob Chemother 2000;46:523–6.
[85] Canepari P, Boaretti M, Del Mae Lleo M, et al. Lipoteichoic acid as a new target for activity of antibiotics: mode of action of daptomycin (LY146032). Antimicrob Agents Chemother 1990;34:1220–6.
[86] Alborn Jr WE, Allen NE, Treston DA. Daptomycin disrupts membrane potential in growing Staphylococcus aureus. Antimicrob Agents Chemother 1991;35:2639–42.
[87] Rybak MJ, Hershberger E, Moldovan T, et al. In vitro activities of daptomycin, vancomycin, linezolid, and quinupristin–dalfopristin against Staphylococci and Enterococci, including vancomycin-intermediate and -resistant strains. Antimicrob Agents Chemother 2000;44:1062–6.
[88] Snydman DR, Jacobus NV, McDermott LA, et al. Comparative in vitro activities of daptomycin and vancomycin against resistant Gram-positive pathogens. Antimicrob Agents Chemother 2000;44: 3447–50.
[89] Pankuch GA, Jacobs MR, Appelbaum PC. Bactericidal activity of daptomycin against *Streptococcus pneumoniae* compared with eight other antimicrobials. J Antimicrob Chemother 2003;51: 443–6.
[90] Wise R, Andrews JM, Ashby JP. Activity of daptomycin against Gram-positive pathogens: a comparison with other agents and the determination of a tentative breakpoint. J Antimicrob Chemother 2001;48:563–7.
[91] Silverman JA, Oliver N, Andrew T, et al. Resistance studies with daptomycin. Antimicrob Agents Chemother 2001;45:1799–802.
[92] Akins RL, Rybak MJ. In vitro activities of daptomycin, arbekacin, vancomycin, and gentamicin alone and/or in combination against glycopeptide intermediate-resistant *Staphylococcus aureus* in an infection model. Antimicrob Agents Chemother 2000;44:1925–9.
[93] Lee BL, Sachdeva M, Chambers HF. Effect of protein binding of daptomycin on MIC and antibacterial activity. Antimicrob Agents Chemother 1991;35:2505–8.
[94] Wise R, Gee T, Andrews JM, et al. Pharmacokinetics and inflammatory fluid penetration of intravenous daptomycin in volunteers. Antimicrob Agents Chemother 2002;46:31–3.
[95] Woodworth JR, Nyhart Jr EH, Brier GL, et al. Single-dose pharmacokinetics and antibacterial activity of daptomycin, a new lipopeptide antibiotic, in healthy volunteers. Antimicrob Agents Chemother 1992;36:318–25.
[96] Louie A, Kaw P, Liu W, et al. Pharmacodynamics of daptomycin in a murine thigh model of *Staphylococcus aureus* infection. Antimicrob Agents Chemother 2001;45:845–51.
[97] Dvorchik B, Sica D, Gehr T. Pharmacokinetics (PK) and safety of single-dose daptomycin in subjects with graded renal insufficiency and end-stage renal disease (ESRD). Program and abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego; 2002. Abst A-1387