

Bactericidal activity of daptomycin against *Streptococcus pneumoniae* compared with eight other antimicrobials

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A spectrum of pneumococci with varying susceptibilities to β -lactams, macrolides and quinolones was tested for susceptibility to nine antibiotics, including the novel lipopeptide daptomycin. Daptomycin was active against all strains (MIC range ≤ 0.5 mg/L; MIC₅₀ 0.125 mg/L; MIC₉₀ 0.25 mg/L). All pneumococci were susceptible to vancomycin, teicoplanin, linezolid and quinupristin/dalfopristin, with MICs < 4.0 mg/L. Time–kill assays with eight strains demonstrated that daptomycin (2 \times MIC) was bactericidal in seven of eight strains tested at 24 h, with $>90\%$ killing at 1 h.

Keywords: pneumococci, daptomycin, MIC, time–kill

Introduction

The prevalence of *Streptococcus pneumoniae* resistant to penicillin G and other antibiotics has increased worldwide at an alarming rate.^{1,2} Antibiotic resistance is a significant concern in the USA. For example, a recent survey has shown that 50.4% of 1476 clinically significant pneumococcal isolates were not susceptible to penicillin.² Furthermore, macrolide resistance was detected in 33% of the isolates, including 5% of penicillin-susceptible, 37% of penicillin-intermediate and 66% of penicillin-resistant strains.² Quinolone-resistant pneumococcal strains have also been reported with increasing frequency, particularly in Hong Kong and Canada.³

Because of this worldwide increase in antibiotic resistance, there is a significant need for additional antimicrobial agents to treat infections caused by penicillin-intermediate and penicillin-resistant pneumococci.^{1,2} Although therapeutic modalities such as β -lactams, macrolides and quinolones are currently available, in view of the increasing resistance described above, an agent with a unique mechanism of action compared with these three antibiotic classes is desirable.

Daptomycin is a novel lipopeptide that exhibits rapid *in vitro* concentration-dependent bactericidal activity against most clinically significant Gram-positive bacteria.^{4–6} Daptomycin is active against antibiotic-susceptible and -resistant

strains, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and antibiotic-resistant pneumococci. The mode of action of daptomycin is unique; it is believed to kill Gram-positive bacteria by disrupting multiple aspects of bacterial plasma membrane function without penetrating into the cytoplasm.^{4,6}

This study was designed to test the activity of daptomycin compared with that of amoxicillin, erythromycin, levofloxacin, linezolid, quinupristin/dalfopristin, penicillin G, teicoplanin and vancomycin against pneumococci using microdilution and time–kill methodologies.

Materials and methods

For MIC analysis, 346 strains of *S. pneumoniae* were tested. Of the 346 strains, 159 were penicillin susceptible (MICs < 0.06 mg/L), 86 were penicillin intermediate (MICs 0.125–1.0 mg/L) and 101 were penicillin resistant (MICs > 2.0 mg/L). Additionally, 207 strains were also erythromycin resistant (MICs > 1.0 mg/L) and 30 were levofloxacin resistant (MICs > 4.0 mg/L). Daptomycin was provided by Cubist Pharmaceuticals, Inc. (Lexington, MA, USA) and the other antimicrobials were obtained from their respective companies, or in the case of penicillin G, from Sigma, Inc. (St Louis, MO,

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USA). Determination of MIC was carried out using micro-dilution techniques recommended by the NCCLS.⁷ Mueller–Hinton broth was adjusted to contain 50 mg of calcium per litre for testing daptomycin.⁴ Previous studies⁴ have documented that the *in vitro* activity of daptomycin is enhanced by increased calcium concentrations, and that MICs thus obtained correlated well with favourable clinical outcome.⁶ This calcium effect is not observed with other agent classes.⁷ The initial calcium concentration in Mueller–Hinton broth was measured by Inductively Coupled Plasma Atomic Spectrometry (ICP), using model 61E ICP (Thermo Jarrell Ash Corporation, Franklin, MA, USA). Frozen microtitre trays containing appropriate concentrations of antibiotics

were prepared by TREK Diagnostic Systems, Inc. (Westlake, OH, USA) and frozen at -70°C until use. Standard quality control strains,⁷ including *S. pneumoniae* ATCC 49619, were included in each experiment.

Time–kill activities were measured in duplicate, as described previously, for eight pneumococcal strains: two were penicillin susceptible and six penicillin resistant (of which three were erythromycin resistant and two levofloxacin resistant).⁸ Mueller–Hinton broth was supplemented as described above, and antibiotic concentrations chosen were $4\times$ and $2\times$ MIC, and the MIC for each strain. Growth controls were included in each experiment.⁸ The initial inoculum for each strain was within the range of 5×10^5 to 5×10^6 cfu/mL.

Table 1. MICs (mg/L) for 346 pneumococcal strains

Drug	MIC range	MIC ₅₀	MIC ₉₀
Daptomycin ^a			
penicillin S	0.06–0.5	0.12	0.25
penicillin I	0.06–0.5	0.12	0.25
penicillin R	0.12–0.5	0.12	0.25
Vancomycin			
penicillin S	0.25–0.5	0.25	0.25
penicillin I	0.12–0.5	0.25	0.25
penicillin R	0.12–0.5	0.25	0.25
Teicoplanin			
penicillin S	≤ 0.016 –0.12	0.03	0.06
penicillin I	≤ 0.016 –0.06	0.03	0.03
penicillin R	≤ 0.016 –0.12	0.03	0.06
Quinupristin/dalfopristin			
penicillin S	0.12–2.0	0.5	0.5
penicillin I	0.12–2.0	0.5	0.5
penicillin R	0.06–2.0	0.5	1.0
Linezolid			
penicillin S	0.5–2.0	1.0	2.0
penicillin I	0.5–2.0	1.0	2.0
penicillin R	0.5–2.0	1.0	2.0
Levofloxacin			
penicillin S	0.5–>8.0	1.0	2.0
penicillin I	0.5–>8.0	1.0	1.0
penicillin R	0.5–>8.0	1.0	>8.0
Penicillin G			
penicillin S	≤ 0.016 –0.06	≤ 0.016	0.03
penicillin I	0.12–1.0	0.5	1.0
penicillin R	2.0–8.0	2.0	4.0
Amoxicillin			
penicillin S	≤ 0.016 –0.12	≤ 0.016	0.03
penicillin I	0.03–2.0	0.25	1.0
penicillin R	1.0–16	2.0	2.0
Erythromycin			
penicillin S	0.03–>16	2.0	>16
penicillin I	0.03–>16	4.0	>16
penicillin R	0.03–>16	>16	>16

S, susceptible; I, intermediate; R, resistant.

^aMueller–Hinton broth contained 50 mg Ca²⁺ per litre for daptomycin testing.

Daptomycin activity against pneumococci

Table 2. Number of strains with indicated rate of killing (%) against eight *S. pneumoniae* strains after indicated time periods

Drug	Rate of killing (%) after											
	1 h			6 h			12 h			24 h		
	90	99	99.9	90	99	99.9	90	99	99.9	90	99	99.9
Daptomycin												
4×MIC	7	4	0	8	8	6	8	8	8	8	8	8
2×MIC	7	2	0	8	8	5	8	8	7	7	7	7
MIC	3	1	0	8	8	4	7	7	6	5	4	4
Linezolid												
4×MIC	0	0	0	4	1	0	8	4	0	8	8	5
2×MIC	0	0	0	1	0	0	6	2	0	7	6	5
MIC	0	0	0	1	0	0	3	1	0	5	3	3
Vancomycin												
4×MIC	1	0	0	8	3	1	8	8	6	8	8	8
2×MIC	0	0	0	8	2	1	8	8	5	8	8	8
MIC	0	0	0	7	2	1	8	8	4	5	4	3
Teicoplanin												
4×MIC	0	0	0	3	0	0	8	5	0	8	8	8
2×MIC	0	0	0	2	0	0	8	4	0	8	8	8
MIC	0	0	0	0	0	0	5	1	0	3	3	3
Quinupristin/dalfopristin												
4×MIC	7	5	2	8	8	7	8	8	8	8	8	8
2×MIC	7	5	2	8	8	6	8	8	8	6	6	6
MIC	5	4	1	7	6	4	7	7	6	4	3	3
Levofloxacin ^a												
4×MIC	0	0	0	6	6	2	6	6	6	6	6	6
2×MIC	0	0	0	6	5	2	6	6	6	6	6	6
MIC	0	0	0	6	3	0	6	6	5	6	6	6
Amoxicillin												
4×MIC	1	0	0	8	4	1	8	8	7	8	8	8
2×MIC	1	0	0	8	3	1	8	8	6	8	8	8
MIC	0	0	0	8	4	0	8	5	4	5	5	5
Erythromycin ^b												
4×MIC	0	0	0	5	1	0	5	5	4	5	5	5
2×MIC	0	0	0	4	0	0	5	4	4	5	5	5
MIC	0	0	0	2	0	0	5	2	1	4	3	2

^aSix strains tested with MIC < 16 mg/L (MIC range 1.0–2.0 mg/L).

^bFive strains tested with MIC < 64 mg/L (MIC range 0.016–0.03 mg/L).

Viability counts of antibiotic-containing cultures were carried out at 0, 1, 6, 12 and 24 h, as described previously.⁸ Colony counts were carried out on plates yielding 30–300 bacterial colonies. The upper limit of sensitivity of colony counts was 300 cfu/mL.⁸

Time–kill assays were analysed by determining the number of strains yielding a $\Delta\log_{10}$ cfu/mL of –1 (90% killing), –2 (99% killing) and –3 (99.9% killing) at 1, 6, 12 and 24 h, compared with counts at 0 h. Results are means of two assays. Bactericidal activity was defined as the lowest antibiotic concentration that reduced the original inoculum by $\geq 3 \log_{10}$ cfu/mL

(99.9%) at each time period, and bacteriostatic activity was defined as a reduction of $< 3 \log_{10}$ cfu/mL in the inoculum concentration.⁸ For time–kill testing with erythromycin, only strains with an erythromycin MIC of ≤ 1.0 mg/L were tested. Additionally, two strains with a levofloxacin MIC of 16 mg/L were excluded from levofloxacin time–kill assays.

Results

Results of MIC testing for the nine antibiotics against the 346 pneumococcal strains are presented in Table 1. Dapto-

mycin, linezolid, vancomycin, teicoplanin and quinupristin/dalfopristin were active against all strains tested irrespective of the strains' susceptibility to β -lactams, macrolides or quinolones. Teicoplanin yielded the lowest MIC₅₀ and MIC₉₀, followed by daptomycin, vancomycin, quinupristin/dalfopristin and linezolid, respectively. The MIC₅₀s and MIC₉₀s of amoxicillin, erythromycin and penicillin G were higher and increased for penicillin-susceptible, -intermediate and -resistant strains. Additionally, levofloxacin was active against the 316 quinolone-susceptible strains, with an MIC₅₀ of 1.0 mg/L and an MIC₉₀ of 2.0 mg/L.

The daptomycin MICs for the eight pneumococcal strains tested in the time–kill assays were similar to those listed in Table 1 and were 0.125 mg/L (three strains), 0.25 mg/L (two strains) and 0.5 mg/L (three strains). Of these, two strains were penicillin susceptible and six were penicillin resistant. Results of the time–kill studies are summarized in Table 2. Results are means of two assays. Daptomycin at 2 \times MIC was bactericidal against seven of eight strains after 24 h, and at 4 \times MIC was bactericidal against all eight strains tested. Significant bacterial killing was observed early, with 90% killing in the seven strains after 1 h of incubation with daptomycin at 2 \times or 4 \times MIC. In comparison, only quinupristin/dalfopristin killed bacteria with similar rapidity after 1 h. Vancomycin, teicoplanin, levofloxacin and amoxicillin were bactericidal against 6–8 strains at 2 \times MIC after 24 h. However, the rate of killing was slower compared with that of daptomycin. In addition, linezolid and erythromycin exhibited poor bactericidal activity compared with all the other agents tested, with bactericidal activity observed in only five of eight strains at 4 \times MIC for both agents.

Discussion

In this study, the *in vitro* activity of daptomycin against pneumococci, measured by MIC determination and time–kill assays, was similar to results described previously.^{4–6} Comparative activities of other agents active against Gram-positive organisms such as vancomycin, linezolid and quinupristin/dalfopristin were similar to previous findings.^{9,10} Interestingly, compared with other antimicrobial agents predominantly active against Gram-positive organisms, daptomycin demonstrated very rapid kill kinetics, with significant killing of pneumococcal strains observed as early as 1 h after antibiotic exposure. In this study, only quinupristin/dalfopristin had comparable bactericidal activity.⁸

This study supports a possible role for daptomycin in treating infections caused by antibiotic-susceptible and -resistant pneumococci, irrespective of the susceptibility of the given

strain to β -lactams, macrolides or quinolones. Further studies are warranted on the bactericidal activity of daptomycin against *S. pneumoniae*, including relationship of kill kinetics with protein binding and pharmacokinetic/pharmacodynamic properties.

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