ANTIMICROBIAL ACTIVITY OF A NOVEL POLYMYXIN ANALOG (CB-182,804) TESTED AGAINST CLINICAL STRAINS OF GRAM-NEGATIVE BACILLI, INCLUDING COLISTIN-RESISTANT ORGANISMS

H.S. SADER, P.R. RHOMBERG, R.N. JONES

Cubist Pharmaceuticals, Inc., Lexington, MA.

ABSTRACT

Background: CB-182,804 is structurally related to polymyxins and has shown rapid bactericidal activity against many Gram-negative (GN) pathogens, including multidrug-resistant (MDR)

Methods: 455 strains selected from various surveillance programs were susceptibility (S) tested by CLSI broth microdilution methods. Strains with acquired resistance (R) to colistin (COL) and/or selected broad-spectrum antimicrobials were included in all organism groups. Organisms tested were: P. aeruginosa (PSA: 100), Acinetobacter spp. (ASP: 81), B. cepacia (BC: 18) S. maltophilia (XM: 25), E. coli (EC; 80), K. pneumoniae (KPN; 81), Citrobacter spp. (CSP; 20), Enterobacter spp. (ESP; 20), indolepositive Proteae (IPP: 10), P. mirabilis (PM: 10) and S. marcescens (SM: 10),

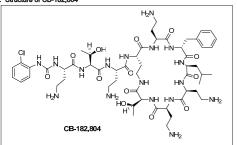
Results: CB-182,804 was very active against PSA (MICso/90, 0.5/2 ug/ml) and ASP (MICso/90, 1/4 μg/ml), including MDR strains. CB-182,804 was also active against E. coli (MICso/90, 1/2 μg/ml) and KPN (MICso/90, 1/4 μg/ml), including ESBL and serine carbapenemase (KPC-2 or 3) producers. When compared to COL, CB-182,804 was more active against PSA (MICso comparisons) and slightly less active against ASP, EC and KPN. CSP had lower MICs for CB-182,804 and COL, while both compounds exhibited variable activity against XM and ESP, and limited activity against BC, IPP, PM

Conclusions: CB-182,804 exhibited in vitro activity and spectrum similar to COL. CB-182,804 was very active against COL-S strains that were R to all other currently available antimicrobials for linical use and may represent and an important therapeutic option for infections caused by an-R GN bacilli, Cross-R with COL was observed

INTRODUCTION

- The continued worldwide emergence of Gram-negative bacteria resistant to most available antimicrobial agents (so called "pan-resistant" organisms) has severely compromised the treatment of bacterial infections and led to increasing use of colistin and polymyxin B as
- Colistin and polymyxin B possess antibacterial activity against a wide variety of Gramnegative pathogens. The great majority of Pseudomonas aeruginosa, Acinetobacter spp., Escherichia coli, Klebsiella spp. and Citrobacter spp., all common and important nosocomial pathogens with increasing antimicrobial resistance, are usually susceptible to the polymyxin
- CB-182,804 (Figure 1) is structurally related to polymyxins and has shown rapid bactericidal activity against many Gram-negative pathogens, including multidrug-resistant (MDR) organisms. In the present study, we evaluated the antimicrobial activity of CB-182.804 tested by reference methods against a collection of clinical Gram-negative bacilli enriched with MDR organisms.

Figure 1. Structure of CB-182.804



METHODS

Bacterial isolates

■ A total of 455 Gram-negative bacilli causing clinical infections submitted to various surveillance programs (JMI Laboratories, North Liberty, IA) were utilized. Tested strains included Enterobacteriaceae (231 strains; 13 species) and non-fermentative Gram-negative bacilli (224 strains; 9 species). These challenge strains were selected to include resistance to colistin, carbapenems and/or broad-spectrum cephalosporins. Strains were primarily collected in 2006 except for a few with very uncommon resistance mechanisms or patterns which were collected in previous years. Species identification was confirmed by standard biochemical tests and or the Vitek Systems (bioMerieux, Hazelwood, MO), when necessary.

■ All isolates were tested for susceptibility using broth microdilution methods as described by the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009) with cation-adjusted Mueller-Hinton broth, CB-182,804 and colistin were tested as well as 12 additional comparator agents. Susceptibility breakpoint interpretations were those found in CLSI documents (M100-S20-U), and quality control (QC) was assured with concurrent testing of E. coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. All QC results were within CLSI specified ranges

Genotypic detection of resistance.

■ Multiplex PCR approaches were used to detect genes encoding plasmidic AmpCs, metallo-β-lactamases (IMP, VIM, SPM, GIM, SIM) and serine-carbapenemases (KPC, IMI/NMC-A, SME, OXA). Amplicons were sequenced and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS

- CB-182,804 (MICso, 0.5 μg/ml and MICso, 2 μg/ml) was very active and slightly more potent than colistin (MIC50, 1 µg/ml and MIC90, 2 µg/ml) when tested against P. aeruginosa, including MDR strains (resistant to carbapenems and/or aminoglycosides and/or
- Acinetobacter spp. strains were very susceptible to CB-182,804 (MIC $_{50}$, 1 μ g/ml and MIC $_{50}$, 4 μ g/ml). CB-182,804 activity against *Acinetobacter* spp. was comparable to that of colistin (MIC50, 0.5 μg/ml and MIC90, 4 μg/ml; Tables 1 and 2).
- CB-182,804 was active against E. coli (MIC50, 1 µg/ml and MIC90, 2 µg/ml) and K. pneumoniae (MIC50, 1 μg/ml and MIC90, 4 μg/ml), including strains producing extendedspectrum β-lactamases (ESBLs) and serine carbapenemases, such as KPC-2 or 3 (Table 3). CB-182,804 was less active than colistin (MIC50/90, 0.25/0.5 µg/ml for E. coli and 0.5/2 µg/ml for K. pneumoniae respectively) against these organisms (Tables 1 and 2).
- Citrobacter spp. strains showed the lowest MIC values for CB-182,804 (MIC $_{90}$, 0.5 μ g/ml) and colistin (MIC $_{90}$, 0.25 μ g/ml; **Tables 1** and 2).
- CB-182,804 and colistin possesed variable activity against Enterobacter spp. and S. maltophilia; while all strains of B. cepacia, indole-positive Proteae, P. mirabilis and S. marcescens showed elevated MIC values for CB-182 804 and colistin (Table 1).
- Table 2 summarizes the in vitro activities of CB-182.804 and all comparator agents evaluated. Resistance to comparator agents were generally high since a great proportion of strains with acquired resistance to colistin and/or carbapenems and/or broad-spectrum cephalosporins were pre-selected for study, and included in all organism groups evaluated.

Table 1. Frequency of occurrence and cumulative percentage inhibited at MIC values for

CB-182,804 and colistin against a large collection of Gram-negative bacilli.

| Organism (no. tested) | ≤0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | >0 |
|------------------------------|---------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| P. aeruginosa (100) | | | | | | | | | | | |
| CB-182.804 | 0 (0.0) | 5 (5.0) | 70 (75.0) | 11 (86.0) | 4 (90.0) | 1 (91.0) | 0 (91.0) | 0 (91.0) | _ | - | 9 (100.0) |
| Colistin | 0 (0.0) | 3 (3.0) | 19 (22.0) | 62 (84.0) | 6 (90.0) | 2 (92.0) | 2 (94.0) | 1 (95.0) | 1 (96.0) | 0 (96.0) | 4 (100.0) |
| Acinetobacter sop. (81) | | | | | | | | | | | |
| CB-182,804 | 1 (1.2) | 5 (7.4) | 18 (29.6) | 39 (77.8) | 9 (88.9) | 3 (92.6) | 2 (95.1) | 2 (97.5) | - | - | 2 (100.0 |
| Colistin | 4 (4.9) | 23 (33.3) | 37 (79.0) | 5 (85.2) | 1 (86.4) | 3 (90.1) | 2 (92.6) | 1 (93.8) | 1 (95.1) | 0 (95.1) | 4 (100.0 |
| B. cepacia (18) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | - | - | 18 (100.0 |
| Colistin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (5.0) | 0 (5.0) | 17 (100.0 |
| S. maltophilia (25) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 1 (4.0) | 3 (16.0) | 0 (16.0) | 3 (28.0) | 2 (36.0) | 5 (56.0) | 4 (72.0) | - | - | 7 (100.0 |
| Colistin | 2 (8.0) | 3 (20.0) | 6 (44.0) | 4 (60.0) | 4 (76.0) | 0 (76.0) | 3 (88.0) | 1 (92.0) | 2 (100.0) | 0 (100.0) | 0 (100.0 |
| E. coli (80) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 1 (1.3) | 9 (12.5) | 42 (65.0) | 23 (93.8) | 0 (93.8) | 0 (93.8) | 1 (95.0) | - | - | 4 (100.0 |
| Colistin | 3 (3.8) | 40 (53.8) | 32 (93.8) | 0 (93.8) | 0 (93.8) | 1 (95.0) | 3 (98.8) | 0 (98.8) | 0 (98.8) | 0 (98.8) | 1 (100.0 |
| K. pneumoniae (81) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 1 (1.2) | 14 (18.5) | 50 (80.3) | 7 (88.9) | 1 (90.1) | 0 (90.1) | 3 (93.8) | - | - | 5 (100.0 |
| Colistin | 0 (0.0) | 37 (46.7) | 30 (82.7) | 5 (88.9) | 1 (90.2) | 0 (90.2) | 3 (93.8) | 3 (97.5) | 2 (100.0) | 0 (100.0) | 0 (100.0 |
| Citrobacter spp. (20) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 1 (5.0) | 18 (95.0) | 1 (100.0) | 0 (100.0) | 0 (100.0) | 0 (100.0) | 0 (100.0) | - | - | 0 (100.0 |
| Colistin | 0 (0.0) | 18 (90.0) | 2 (100.0) | 0 (100.0) | 0 (100.0) | 0 (100.0) | 0 (100.0) | 0 (100.0) | 0 (100.0) | 0 (100.0) | 0 (100.0 |
| Enterobacter spp. (20) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 0 (0.0) | 5 (25.0) | 9 (70.0) | 1 (75.0) | 0 (75.0) | 0 (75.0) | 0 (75.0) | - | - | 5 (100.0 |
| Colistin | 0 (0.0) | 13 (65.0) | 2 (75.0) | 0 (75.0) | 0 (75.0) | 1 (80.0) | 0 (80.0) | 0 (80.0) | 1 (90.0) | 1 (95.0) | 2 (100.0 |
| Indole-positive Proteae (10) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | - | - | 10 (100.0 |
| Colistin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 10 (100.0 |
| P. mirabilis (10) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | - | - | 10 (100.0 |
| Colistin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 10 (100.0 |
| S. marcescens (10) | | | | | | | | | | | |
| CB-182,804 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | - | - | 10 (100.0 |
| Colistin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 10 (100.0 |

Table 2. Activity of CB-182,804 and comparator antimicrobial agents when tested against Gram-

- = dilution not teste

| Organism/Antimicrobial agent | MIC ₅₀ | MIC _{so} | Range | %Susceptible %Resistant* |
|------------------------------|-------------------|-------------------|-----------|-----------------------------|
| Pseudomonas aeruginosa (100) | | | | |
| CB-182,804 | 0.5 | 2 | 0.25->16 | -/- |
| Colistin | 1 | 2 | 0.25->64 | 90.0 / 8.0 |
| Meropenem | 2 | >32 | 0.06->32 | 57.0 / 28.0 |
| Ceftazidime | 8 | >16 | ≤1->16 | 51.0 / 44.0 |
| Piperacillin/tazobactam | 16 | >64 | ≤0.5->64 | 67.0 / 33.0 |
| Amikadin | 8 | >32 | ≤4->32 | 66.0 / 32.0 |
| Ciprofloxacin | 1 | >4 | ≤0.03->4 | 51.0 / 42.0 |
| Acinetobacter spp. (81) | | | | |
| CB-182,804 | 1 | 4 | 0.12->16 | -/- |
| Colistin | 0.5 | 4 | 0.12->64 | 86.4 / 13.6 |
| Meropenem | 1 | 32 | 0.06->32 | 69.1 / 28.4 |
| Ceftazidime | 16 | >16 | ≤1->16 | 46.9 / 48.1 |
| Cefepime | 8 | >16 | ≤0.12->16 | 51.9 / 30.9 |
| Piperacillin/tazobactam | 16 | >64 | ≤0.5->64 | 51.9 / 30.9 |
| Amikadin | ≤4 | >32 | ≤4->32 | 69.1 / 24.7 |
| Ciprofloxacin | 1 | >4 | ≤0.03->4 | 50.6 / 49.4 |
| Tigecycline | 0.5 | 2 | ≤0.03-4 | -/- |

Table 3. Activity of CB-182,804 and selected comparator agents tested against β-lactamase producing Gram-negative organisms.

RESULTS

Sprofloxacin Tigecycline

CB-182 804

Amikacin

CB-182,804 Colistin Meropenem

Ceftazidime Piperacillin/ta: Amikacin Ciprofloxacin Tigecycline^b

Amikacin Ciprofloxacin Tigecycline^b Citrobacter spp. (20) CB-182,804 Colistin Meropenem Ceftazidime

Ceftazidime
Piperacillin/tazobact
Amikacin
Ciprofloxacin
Tigecycline^b
interobacter spp. (20)
CB-182,804
Colistin

Meropenem Ceftazidime Piperacillin/ti Amikacin

Ciprofloxacin Tigecycline^b dole-positive Pr

CB-182.804

Colistin Meropenem Ceftazidime

Piperacillin/ta: Amikacin Ciprofloxacin Tigecycline

roteus mirabilis CB-182,804

Meropenem Ceftazidime Piperacillin/ts Amikacin

CB-182,804 Colistin Meropenem Ceftazidime

Ciprofloxacin Tigecycline terratia marcescens (1

US-FDA breakpoints were applied [Tygacil Product Insert, 2009].

c. Breakpoints established by the CLSI for P. aeruginosa were applied for comparison purpose only ICLSI 2010.

Ciprofloxacin Tigecycline ischerichia coli (8)

0.06-16 \$1->16 1->16 \$0.5->64

S1->16

0.06-1

≤0.015->32

54->32

0.25-1

≤0.015-0.12 ≤1->16

0.12-0.5

0.5->16 0.25->64 s0.015-16 s1->16 1->64

s0.5-4 s4-8 s0.03->4

0.03-0.06 ≤1-4 ≤0.5-2

88.9 / 11.1

76 0 / 24 0

75.0 / 22.5

100 0 / 0 0

84.0 / 4.9

100 0 / 0 05

50.0 / 50.0

75.0 / 5.0

100.0 / 0.0

75.0 / 20.0

95.0 / 5.0

100.0 / 0.0

85.0 / 15.0 100.0 / 0.0

100.0 / 0.0

100.0 / 0.0

0.0 / 100.0°

90.0 / 0.0

50.0 / 50.0

| Organism (no. tested)/ Antimicrobial agent | MIC ₅₀ | MIC ₉₀ | MIC range | %S/%R |
|---|-------------------|-------------------|-----------|-------------|
| P. aeruginosa (19)* | | | | |
| CB-182,804 | 0.5 | 1 | 0.5->16 | -/- |
| Colistin | 1 | 2 | 0.5-8 | 94.7 / 5.3 |
| Meropenem | >32 | >32 | 16->32 | 0.0 / 100.0 |
| Ceftazidime | >16 | >16 | >16 | 0.0 / 100.0 |
| A. baumanni (10)† | | | | |
| CB-182,804 | 1 | 1 | 0.5-2 | -/- |
| Colistin | 0.5 | 0.5 | 0.25-0.5 | 100.0 / 0.0 |
| Meropenem | 16 | >32 | 16->32 | 0.0 / 100.0 |
| Ceftazidime | >16 | >16 | >16 | 0.0 / 100.0 |
| E. coli (12)* | | | | |
| CB-182,804 | 1 | 2 | 0.25-2 | -/- |
| Colisitin | 0.5 | 0.5 | 0.12-0.5 | 100.0 / 0.0 |
| Meropenem | 2 | 4 | 0.03-8 | 66.7 / 25.0 |
| Ceftazidime | 16 | >16 | 2->16 | 16.7 / 83.3 |
| K. pneumoniae (10) | | | | |
| CB-182,804 | 1 | 1 | 0.5-1 | -/- |
| Colistin | 0.5 | 0.5 | 0.25-0.5 | 100.0 / 0.0 |
| Meropenem | 16 | >32 | 1->32 | 10.0 / 90.0 |
| Ceftazidime | >16 | >16 | >16 | 0.0/ 100.0 |

Includes strains producing KPC-2/3 (7), plasmidic AmpC enzyme Includes strains producing KPC (6) and VIM (4) carbapenemases

CONCLUSIONS

- CB-182,804 exhibited in vitro potency and a spectrum of activity most similar to that of colistin. CB-182,804 was active against colistin susceptible (MIC, ≤2 µg/ml) P. aeruginosa and Acinetobacter spp., including MDR strains.
- CB-182,804 was also active against E. coli, K. pneumoniae, Citrobacter spp., and Enterobacter spp. isolates including MDR strains. However, isolates with elevated colistin MIC results also exhibited elevated MIC values for CB-182.804.
- CB-182,804 showed limited activity against organisms intrinsically resistant to colistin, such as indole-positive Proteae, P. mirabilis and S. marcescens.
- CB-182,804 was active against colistin-susceptible strains that were resistant to all other currently available antimicrobials for clinical use, and this new agent may represent an important therapeutic option for infections caused by pan-resistant Gram-negative bacilli.

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