Journal of Antimicrobial Chemotherapy (2004) **53**, 804–807
DOI: 10.1093/jac/dkh184
Advance Access publication 31 March 2004

JAC

Potential utility of a peptide deformylase inhibitor (NVP PDF-713) against oxazolidinone-resistant or streptogramin-resistant Gram-positive organism isolates

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Received 15 December 2003; returned 20 January 2004; revised 8 February 2004; accepted 13 February 2004

***Objectives*: To evaluate the potency of a novel peptide deformylase inhibitor, NVP PDF-713, against Gram-positive organisms having resistances to linezolid or quinupristin/dalfopristin.**

***Materials and methods*: A total of 45 strains from three genera (six species groups) were tested by reference broth microdilution methods. The mechanism of resistance to the oxazolidinone was determined by sequencing of the gene encoding the ribosomal target.**

***Results*: NVP PDF-713 retained activity against linezolid-resistant staphylococci (MIC range 0.25–2 mg/L), *Streptococcus oralis* (MIC 0.5 mg/L), *Enterococcus faecalis* (MIC range 2–4 mg/L) and *Enterococcus faecium* (MIC range 0.5–4 mg/L). Quinupristin/dalfopristin-resistant *E. faecium* (MIC range 1–2 mg/L) and staphylococci (MIC range 0.12–2 mg/L) were also inhibited by NVP PDF-713. Many (10 of 13 strains) of the linezolid-resistant enterococci were resistant to vancomycin and these clinical strains had a G2576U ribosomal target mutation.**

***Conclusions*: NVP PDF-713 appears to be a promising clinical candidate among the peptide deformylase inhibitors for the treatment of infections caused by Gram-positive organisms that possess resistances to oxazolidinones or streptogramin combinations.**

Keywords: streptococci, enterococci, staphylococci, streptogramins, oxazolidinones

Introduction

Numerous new and novel antimicrobial agents have been introduced into infectious disease practice in the last decade to address emerging resistances among Gram-positive cocci.[1–3] Resistances to oxazolidinones,[2,3] streptogramin combinations[1] and various glycopeptides[2] require expanded development of agents with alternative targets or modes of action. Peptide deformylase, a required enzyme for prokaryote protein synthesis, has been suggested[4,5] as a potential target for inhibitors such as the potent hydroxamic acid derivatives.[6] The concept has been validated[7,8] and several candidate agents have been screened.[7,8] During initial development, resistance mechanisms were also described among multiple Gram-positive organisms including *Staphylococcus aureus*.[9–12]

In this investigation, NVP PDF-713, a new peptide deformylase inhibitor from a novel series of compounds,[13] was tested—using reference susceptibility test methods—[14,15]against a collection of recent clinical isolates having documented resistances to linezolid or quinupristin/dalfopristin.

Materials and methods

Bacterial strains

A total of 45 organisms, originally isolated at resistance surveillance sites in the USA, Canada, Brazil and Europe, were selected from the stock culture collection (2001–2002) of JMI Laboratories (North Liberty, IA, USA). These organisms included *Enterococcus faecalis* (linezolid-resistant, three strains; quinupristin/dalfopristin resistance was intrinsic), *Enterococcus faecium* (linezolid-resistant, 10 strains; quinupristin/dalfopristin-resistant, six strains), *S. aureus* (linezolid-resistant, five strains; quinupristin/dalfopristin-resistant, 10 strains), coagulase-negative staphylococci (linezolid-resistant, one strain; quinupristin/dalfopristin-resistant, nine strains) and *Streptococcus oralis* (linezolid-resistant, one strain). Definitions of resistance were those published by the NCCLS.[14,15]

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Table 1. Activity of NVP PDF-713 tested against 19 isolates of linezolid- or quinupristin/dalfopristin-resistant *Enterococcus* spp.

Organism (no. tested)	MIC (mg/L)			
	NVP PDF-713	linezolid	quinupristin/dalfopristin	vancomycin
Linezolid-resistant strains (13)[a]				
E. faecalis				
15–5341	4	8	8	16
04–2V	4	16	8	>32
21–6943A	2	8	8	>32
E. faecium				
17–982A	1	16	0.5	0.5
24–1575A	1	16	1	1
15–4011A	0.5	8	1	16
11–4103A	2	8	0.5	>32
17–14203A	1	16	1	>32
84–7093A	2	16	1	>32
Chicago-1	1	8	0.25	>32
Chicago-2	4	8	1	>32
Chicago-4	4	8	1	>32
Chicago-5	1	32	0.5	0.5
Streptogramin-resistant strains (6)				
E. faecium				
2–12989A	2	2	4	1
38–6823A	2	2	8	4
38–11656A	2	2	8	1
42–13111A	1	2	8	4
65–1208A	1	1	>8	2
103–13202A	1	1	4	2
MIC range (mg/L)	0.5–4	8–32[b]	4–>8[b]	0.5–>32
Median (mg/L)	1	8[b]	8[b]	16

[a]All linezolid-resistant isolates had a documented G2576U mutation.
[b]Resistant subsets only.

Susceptibility testing

All susceptibility tests were performed using NCCLS M7-A6 methods[14] with 2%–5% lysed horse blood supplement for the fastidious streptococci. Cation-adjusted Mueller–Hinton broth was used for all other tested species. The mechanisms of resistance for all linezolid-resistant strains (MICs ≥ 8 mg/L) were confirmed by gene sequencing of the ribosomal target[3] and the detection of a G2576U mutation. The MICs of quinupristin/dalfopristin for quinupristin/dalfopristin-resistant strains were phenotypically confirmed by disc diffusion and Etest (AB Biodisk, Solna, Sweden) to have an MIC at ≥4 mg/L. PCR tests for *vatD* and *vatE* were negative.[1]

Quality control (QC) of the NVP PDF-713 MIC results was performed using acceptable MIC ranges reported by Anderegg *et al.*[16] for QC strains *S. aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619 and *E. faecalis* ATCC 29212. All QC results for NVP PDF-713[16] and comparison agents used to categorize resistant isolates (linezolid, quinupristin/dalfopristin, vancomycin) were within NCCLS[15] published limits. Trays were manufactured by TREK Diagnostics (Cleveland, OH, USA) to specified NCCLS standards.[14] The proposed or tentative susceptible breakpoint for NVP PDF-713 to be applied by clinical trial laboratories was ≤8 mg/L based on pharmacokinetic/pharmacodynamic characteristics of this compound and similar peptide deformylase inhibitors.[17]

Results and discussion

Table 1 lists the results for all linezolid- or quinupristin/dalfopristin-resistant enterococci. Among the 13 linezolid-resistant strains, three were *E. faecalis* and 10 were *E. faecium*. Also, 10 enterococci were resistant to vancomycin, and all *E. faecalis* strains had the characteristic intrinsic streptogramin resistance (MICs 8 mg/L). The MICs of NVP PDF-713 for these enterococci were in the range 0.5–4 mg/L (MIC_{90} 4 mg/L). The six quinupristin/dalfopristin-resistant *E. faecium* isolates were susceptible to vancomycin (MICs 1–4 mg/L) and also inhibited by 1 or 2 mg/L of NVP PDF-713.

Similarly in Table 2, NVP PDF-713 was highly active against linezolid-resistant *S. aureus* (MICs 0.25–0.5 mg/L), *Staphylococcus epidermidis* (MIC 2 mg/L) and the viridans group streptococcus isolate (MIC 0.5 mg/L), i.e. seven strains. Quinupristin/dalfopristin and vancomycin were also active against these oxazolidinone-resistant organisms. The quinupristin/dalfopristin-resistant staphylococci (19 strains) were susceptible to NVP PDF-713 (MIC range 0.12–2 mg/L), linezolid (MICs 1 or 2 mg/L) and vancomycin (MICs 1 or 2 mg/L).

These results indicate that NVP PDF-713, among the new candidate peptide deformylase inhibitors,[13] demonstrates excellent activity (all MICs ≤ 4 mg/L) against emerging Gram-positive clinical isolates that have become resistant to oxazolidinones (linezolid and

Table 2. Activity of NVP PDF-713 tested against 26 isolates of linezolid- or quinupristin/dalfopristin-resistant staphylococci or streptococci

Organism (no. tested)	MIC (mg/L)			
	NVP PDF-713	linezolid	quinupristin/dalfopristin	vancomycin
Linezolid-resistant strains (7)[a]				
S. aureus				
106–12591A	0.5	32	0.5	1
BZ-2	0.25	16	0.5	1
BZ-3	0.5	16	0.5	2
PF-3839	0.5	>32	0.25	2
PF-3840	0.25	>32	0.25	2
S. epidermidis				
82–1645A	2	32	0.25	2
S. oralis				
27–2832A	0.5	32	0.5	0.5
Streptogramin-resistant strains (19)				
S. aureus				
61–4725C	1	1	8	1
61–7949C	0.25	1	>8	2
61–10880A	1	1	8	1
90–2728C	0.5	1	8	1
90–11371A	0.5	2	4	1
91–2220C	1	1	>8	2
91–2811C	1	1	8	2
300–12053A	1	2	>8	1
301–4244A	1	1	8	2
301–10721A	0.5	2	4	1
Coagulase-negative staphylococci				
48–11901A	0.12	2	4	1
53–8021A	1	1	4	2
57–4260A	0.25	2	8	1
63–5921A	1	1	>8	1
78–5445A	2	1	4	2
78–7937A	2	1	8	2
78–13608A	2	1	>8	2
90–10840A	0.5	1	>8	1
300–15319A	2	1	4	2
MIC range (mg/L)	0.12–2	16–>32[b]	4–>8[b]	0.5–2
Median (mg/L)	0.5	32[b]	8[b]	1, 2

[a]All linezolid-resistant isolates had a documented G2576U mutation.
[b]Resistant subsets only.

AZD-2563) or streptogramin combinations.[1,3] Since numerous members of this new peptide deformylase inhibitor class[4–7] may be advanced into clinical trials, close surveillance should be initiated for 'inhibitor-resistant' isolates, as predicted by early molecular studies.[9–12] Continued development of agents in this class seems prudent and further synthetic modifications could enhance potency and other microbiological features, particularly against some Gram-negative species.

Acknowledgements

We thank the technical staff at JMI Laboratories for their excellent testing, and the following individuals for manuscript/editorial/analysis support: K. Meyer, M. Beach, D. Biedenbach, P. Rhomberg and G. Deshpande. This study was supported by an educational/research grant from Novartis Pharmaceuticals, Inc.

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