Peptides 91 (2017) 8–12

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Structure–activity studies on polymyxin derivatives carrying three positive charges only reveal a new class of compounds with strong antibacterial activity

Martti Vaara [a,b,c,*], Timo Vaara [a], Jonathan M. Tyrrell [d]

[a] Northern Antibiotics Ltd., FI-02150 Espoo, Finland
[b] Division of Clinical Microbiology, Helsinki University Hospital, FI-00029 Helsinki, Finland
[c] Department of Bacteriology and Immunology, Helsinki University Medical School, FI-00014 Helsinki, Finland
[d] Department of Medical Microbiology & Infectious Disease, Institute of Infection & Immunity, UGW Main Building, Heath Park Campus, Cardiff, Wales, UK

ARTICLE INFO

Article history:
Received 2 February 2017
Received in revised form 6 March 2017
Accepted 7 March 2017
Available online 11 March 2017

Keywords:
NAB739, NAB815
Escherichia coli
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
Excretion to urine
Nephrotoxicity
HK-2

ABSTRACT

Recent years have brought in an increased interest to develop improved polymyxins. The currently used polymyxins, i.e. polymyxin B and colistin (polymyxin E) are pentacationic lipopeptides that possess a cyclic heptapeptide part with three positive charges, a linear "panhandle" part with two positive charges, and a fatty acyl tail. Unfortunately, their clinical use is shadowed by their notable nephrotoxicity. We have previously developed a polymyxin derivative NAB739 which lacks the positive charges in the linear part. This derivative is better tolerated than polymyxin B in cynomolgus monkeys and is, in contrast to polymyxin B, excreted into urine in monkeys and rats. Here we have conducted further structure-activity relationship (SAR) studies on 17 derivatives with three positive charges only. We discovered a remarkably antibacterial class, as exemplified by NAB815, that carries two positive charges only in the cyclic part.

1. Introduction

Recent years have brought in an increased interest to develop improved polymyxins for the therapy of severe infections caused by extremely antibiotic-resistant Gram-negative bacteria, as reviewed by Pirri et al. [10], Vaara [12], Kadar et al. [5] Cochrane and Vederas [2] and Velkov et al. [19].

The currently used polymyxins, i.e. polymyxin B and colistin (polymyxin E) were discovered as early as 1947. They are pentacationic lipopeptides that possess a cyclic heptapeptide part with three positive charges, a linear "panhandle" part with two positive charges, and a fatty acyl tail (methyloctanoyl in polymyxin B1 and colistin A, or methylheptanoyl in polymyxin B2 and colistin B). For the structure of polymyxin B, see Fig. 1.

Polymyxin B and colistin (administered as its inactive methanesulphonate prodrug CMS) are effective against most species of Enterobacteriaceae as well as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* but both were largely abandoned in the sixties because of nephrotoxicity and increased availability of more effective antibiotics [4–6,12]. According to recent studies, the nephrotoxicity rate of polymyxin B and colistin varies from 20% to 60%. Furthermore, pharmacokinetic data indicates that the current dosage regimens are suboptimal [3]. Therefore, larger doses should be used, but this further increases the risk of nephrotoxicity.

We have previously developed polymyxin derivatives such as NAB739 (Table 1) which lack the positive charges in the linear part and accordingly carry three positive charges only [12–15]. In contrast to the old polymyxins that are effectively reabsorbed by the proximal tubular kidney cells and not excreted as a biologically active form into urine, NAB739 and compounds related to it yield very high concentrations in the urine [12,14,17]. This is remarkable, since most of the bacteremic infections caused by Enterobacteriaceae originate from complicated urinary tract infections. NAB739 has reduced affinity for the brush border membrane of the proximal tubular cells and was shown to be less toxic than polymyxin B to these cells by a factor of more than 20 [13,16]. Preclinical studies on NAB739 are ongoing.

* Corresponding author at: Northern Antibiotics Ltd., Technopolis, Tekniikantie 14, FI-02150 Espoo, Finland.
E-mail address: martti.vaara@northernantibiotics.com (M. Vaara).

http://dx.doi.org/10.1016/j.peptides.2017.03.002
0196-9781/

Table 1
Structure of the compounds, their activity against *E. coli*, and their toxicity to HK-2 cells.[a]

Compound	R (FA)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	MIC (μg/ml)[b] for *E. coli* AT25922	MIC (μg/ml)[b] for *E. coli* IH3080	IC_{50} (μg/ml)[c] for HK-2 cells
Polymyxin B	MOA/MHA	-Dab$^+$	-Thr	-Dab$^+$	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Dab$^+$	-Dab$^+$	-Thr]	**0,5 (0,5-1) [15]**	**0,5 (0,5-1) [15]**	18,0 (7,6 - 38) [4]
NAB739	OA	-	-Thr	-DSer	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Dab$^+$	-Dab$^+$	-Thr]	**2 (1-4) [30]**	**2 (1-4) [30]**	**237 (184-275) [3]**
NAB807	OA	-	-Thr	-DThr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Dab$^+$	-Dab$^+$	-Thr]	4 (2-4) [6]	4 (4-8) [6]	**235**
NAB809	OA	-	-Thr	-DHSer	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Dab$^+$	-Dab$^+$	-Thr]	16 [3]	16 (16-32) [3]	-
NAB743	OA	-	-Thr	-Ser	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Dab$^+$	-Dab$^+$	-Thr]	≥ 32 [3]	16 [3]	**113**
NAB805	OA	-	-Thr	Thr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Dab$^+$	-Dab$^+$	-Thr]	≥ 32 [3]	≥ 32 [3]	**221**
NAB803	OA	-	-Thr	HSer	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Dab$^+$	-Dab$^+$	-Thr]	≥ 32 [3]	≥ 32 [3]	**317**
NAB813	OA	-Dab$^+$	-Thr	-DSer	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Abu	-Dab$^+$	-Thr]	**2 [6]**	**2 [6]**	-
NAB819	NA	-Dab$^+$	-Thr	-DSer	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Abu	-Dab$^+$	-Thr]	**2 [3]**	**2 [3]**	-
NAB812	OA	-Dab$^+$	-Thr	-DSer	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Ser	-Dab$^+$	-Thr]	**2 [3]**	**2 (1-2([3]**	-
NAB818	OA	-Dab$^+$	-Thr	-DSer	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Ser	-Dab$^+$	-Thr]	**2 [3]**	**2 (1-2([3]**	-
NAB815	OA	-Dab$^+$	-Thr	-DThr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Abu	-Dab$^+$	-Thr]	**2 (2-4) [24]**	**2 (2-4) [24]**	**334**
NAB820	NA	-Dab$^+$	-Thr	-DThr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Abu	-Dab$^+$	-Thr]	4 (2-4) [3]	**2 [3]**	-
NAB814	OA	-Dab$^+$	-Thr	-DThr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Ser	-Dab$^+$	-Thr]	**2 [3]**	**2 [3]**	-
NAB821	OA	-Dab$^+$	-Thr	-DThr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Leu	-Dab$^+$	-Thr]	4 (4-8) [3]	4 (4-8) [3]	-
NAB816	OA	-	-Dab$^+$	-DThr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Abu	-Dab$^+$	-Thr]	> 64 [3]	> 64 [3]	-
NAB817	OA	-	-Thr	-Dap$^+$	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Abu	-Dab$^+$	-Thr]	16 [3]	8 [3]	-
NAB822	OA	-Dab$^+$	-Thr	-DThr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Abu	-Dab$^+$	-Leu]	64 (64 - >64) [3]	8 [3]	-
NAB823	OA	-Dab$^+$	-Thr	-DThr	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Leu	-Dab$^+$	-Leu]	16 (16-32) [3]	8 [3]	-
NAB824	OA	-	-Thr	-DSer	-cy[Dab	-Dab$^+$	-DPhe	-Leu	-Dab$^+$	-Dab$^+$	-Leu]	32 (16-32) [3]	16 [3]	56

[a] *Abbreviations:* MOA/MHA, a mixture of methyloctanoic and methylheptanoic acid; OA, octanoic acid; NA, nonanoic acid; Abu, aminobutyric acid; Dab, diaminobutyric acid; Fser, homoserine; cy indicates the R4-R10 ring.
[b] Median, range (in parentheses), number of determinations (in brackets). Median values <4 in **bold**.
[c] Geometric mean, range (in parentheses), number of determinations (in brackets). Values >100 in **bold**.