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The polyamino-isoprenic efflux inhibitor NV716 revives old disused antibiotics against intracellular forms of infection by *Pseudomonas aeruginosa*

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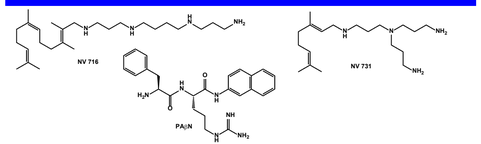
Introduction & Purpose

WHO considers *P. aeruginosa* (PA) as a priority pathogen for the search of innovative therapies. PA is indeed intrinsically resistant to many antibiotics due to poor outer membrane permeability and/or active efflux [1]. Moreover, it can also adopt specific lifestyles, like intracellular survival, which are poorly responsive to antibiotics [2].

Our aim was to evaluate the capacity of efflux pump inhibitors to restore the activity of old, disused antibiotics, against intracellular PA. We compared PAβN to original polyamino-isoprenic compounds, namely NV731 and NV716 in combination with doxycycline, chloramphenicol (substrates for efflux), and rifampicin (not substrate) [3].

chemical structure of inhibitors



References

1. Krishnamoorthy et al. mBio. 2017, 8 : 1172-17.
2. Ribet, et al. Microbes and Infection. 2015, 17:173-83.
3. Borselli et al. Plos One. 2016, 11: e0154490.
4. Buyck et al. Antimicrob Agents Chemother . 2013, 57: 2310-18.

Acknowledgments

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This poster will be made available after the meeting at *http://www.facm.ucl.ac.be/posters.htm*

Results

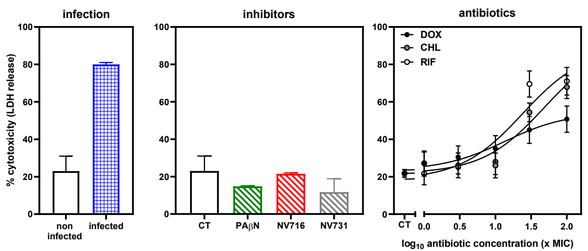
MIC of ABs alone or combined with inhibitors against PAO1

Experimental condition	MIC (mg/L)[a] DOX	CHL	RIF
Antibiotic (AB) alone	8	32	16
AB + NV716 (2.5μM)	**1**	**2**	**0.25**
AB + NV716 (10μM)	**0.5**	**1**	**0.125**
AB + NV731 (2.5μM)	4	32	16
AB + NV731 (10μM)	4	16	8
AB + PAβN (20mg/L)	**2**	**8**	8

[a] values in bold denote a decrease of ≥ 2 doubling dilutions vs. AB alone

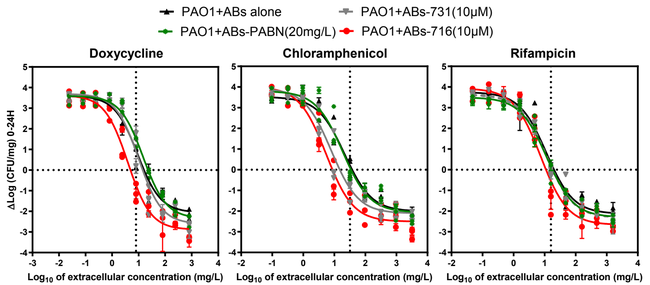
- **PAβN** decreased the MICs of doxycycline and chloramphenicol but not that of rifampicin
- NV731 had no significant effect on MICs
- **NV716 (2.5 and 10 μM)** markedly reduced the MIC of all antibiotics.

Cytotoxicity of ABs/inhibitors in this model



- Infection caused high cell mortality at 24 h
- **PABN, 716, 731** alone were not toxic
- Antibiotics were cytotoxic at high concentrations, but not more in combination with inhibitors, neither in infected cells (not illustrated)

Concentration-response curves for ABs alone or combined with inhibitors against intracellular PAO1



- For antibiotics alone, C_s were close to their MIC in broth and E_{max} were close to 2 log decrease in cfu.
- NV731 and PAβN did not modify these parameters
- **NV716 (10μM)** was able to increase both relative potency (lower C_s value) and maximal efficacy (more negative E_{max} value) for all drugs.

Pharmacodynamic parameters of ABs against intracellular PAO1

Experimental condition	C_s[b] (mg/L) DOX	CHL	RIF	E_{max}[c] (Δlog cfu from post-phagocytosis inoculum) DOX	CHL	RIF
Antibiotic (AB) alone	16.0±8.2	37.5±9.3	20.2±4.0	-2.1±0.2	-2.0±0.3	-2.1±0.3
AB + NV716 (2.5μM)	17.9±3.7	42.1±7.8	19.7±3.5	-2.4±0.2	-1.9±0.3	-2.2±0.3
AB + NV716 (10μM)	4.9±1.2*	8.7±5.3*	11.1±2.3*	-2.9±0.5*	-2.5±0.3*	-2.7±0.4*
AB + NV731 (2.5μM)	16.3±1.7	41.1±8.2	26.2±1.0	-2.6±0.2	-2.1±0.2	-2.4±0.4
AB + NV731 (10μM)	14.2±4.0	15.0±9.1*	18.2±3.8	-2.6±0.4	-2.1±0.2	-2.3±0.4
AB + PAβN (20mg/L)	22.9±1.0	34.5±13.8	17.9±1.2	-2.3±0.2	-2.1±0.3	-2.3±0.3

[b] Cs: apparent static concentration i.e., the extracellular concentration (mg/L) resulting in no apparent bacterial growth (number of cfu identical to the initial [extracellular] or post-phagocytosis [intracellular] inoculum)
[c] Emax: relative maximal efficacy: maximal decrease in inoculum (in $\log_{10}$ units) compared to the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration.
* significantly different ($p < 0.05$) from AB alone (1 way ANOVA with Tukey's Multiple Comparison Test)

Methods

- Phagocytosis of PAO1 (opsonized with human serum) was allowed for 2 hours using a bacterium:THP-1 cell ratio of 10:1, after which non-phagocytosed bacteria were eliminated by incubation with gentamicin (50 X MIC) for 1 hour. After washing, infected cells were incubated with antibiotics (0.003-100 x MIC) for 24 hours.
- Maximal relative efficacy (E_{max}) and apparent static concentrations (C_s) were calculated using the Hill equation of concentration-response curves.
- Toxicity towards THP-1 cells was assessed by measuring the release of the cytosolic enzyme lactate dehydrogenase (LDH) in the culture medium [4].

Main messages

- In contrast to PAβN that act as an efflux inhibitor against planktonic bacteria only, NV716 is capable to re-sensitize PA to antibiotics whether substrates (doxycycline, chloramphenicol) or not (rifampicin) for efflux, not only in broth but also intracellularly. This could be due to its capacity to impair PA membrane integrity [3].
- NV716 may therefore appear as a useful adjuvant to revive the activity of old antibiotics with low antipseudomonal activity against PA infections, including its intracellular persistent forms.