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Rx-P873, a novel protein synthesis inhibitor, accumulates in human THP-1 monocytes and is active against extracellular and intracellular *Pseudomonas aeruginosa (Pa)*.

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Abstract (revised)

Background: The pyrrolocytosine RX-P873 inhibits translation by stabilizing a distorted binding mode of P-site tRNA (ICAAC 2011; F1-1842). Its broad-spectrum includes MDR *Pa* (AAC 2012; 56:1646-9). Intracellular survival contributes to persistence of *Pa* infections. We studied the accumulation of RX-P873 in human THP-1 cells and its extracellular and intracellular activity against sensitive and MDR *Pa*, compared to CAZ and CIP (effective against intracellular *Pa* [AAC 2013; 57:2310-8]).
Methods: MICs were measured by microdilution (CLSI). Extracellular and intracellular activities (24 h) were assessed in broth and THP-1 cells using a PD model (AAC 57:2310-8) for determination of apparent static concentrations (C_s) and relative efficacies at 20 mg/L or at 10xMIC ($E_{20mg/L}$; E_{10xMIC}). Concentration in cells was assayed by fluorimetry (λ_{exc} = 280 nm; λ_{em} = 450nm).
Results: RX-P873 MICs were equal for PAO1 and PA256 (MDR). Against 8 SCVs, RX-P873 MICs were 0.5-2 mg/L (similar to PAO1) while CIP MICs were 2-4 mg/L (*vs.* 0.125 mg/L for PAO1). Extracellularly, all drugs were static at concentrations close to their MIC and cidal at 10X MIC. Intracellularly, C_s were close to MICs for all drugs but efficacy was reduced, with only RX-P873 causing > 1.5 log reduction against all strains at 20 mg/L. E_{10xMIC} was comparable for all drugs against PAO1 or PA256. At extracellular concentrations ranging from 5 to 20 mg/L, RX-P873 accumulated 4-6 times in THP-1 cells after 2 h.
Conclusion: RX-P873 shows similar activity against normal phenotype and SCV of *Pa*, and is not affected by MDR to current drugs. While accumulating only moderately in THP-1 cells, it is highly effective against intracellular *Pa*, including a MDR strain. This suggests a potential interest of RX-P873 for intracellular infection by Gram(-) bacteria resistant to current drugs.

References

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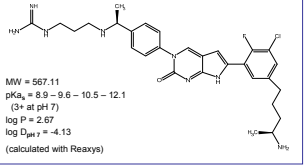
This poster will be made available after the meeting at http://www.facm.ucl.ac.be/posters

Background

Pseudomonas aeruginosa is an opportunistic pathogen responsible for severe pulmonary infections in debilitated patients hospitalized in ICU or suffering from cystic fibrosis. Infections caused by *P. aeruginosa* are difficult to treat due to (i) the remarkable ability of the pathogen to express constitutive and inducible resistance mechanisms (low permeability of the outer membrane, efflux pumps [over]expression, enzymatic inactivation of antibiotics) and (ii) its ability to enter and survive in eucaryotic cells, where the efficacy of antibiotics is considerably reduced [1].
Discovering novel antibacterial agents acting on unexploited targets is critically needed to cope with infections caused by multiresistant organisms [2]. Exploring their cellular pharmacokinetics and pharmacodynamics may help to correctly position them for the treatment of persistent or recurrent infections where intracellular survival may play an important role.
The pyrrolocytosine RX-P873 is a novel antibiotic currently under development by Rib-X Pharmaceuticals. It inhibits protein synthesis by a new mode of action (inhibition of translation by stabilizing a distorted binding mode of P-site tRNA [3]) and presents potent *in vitro* activity against a number of Gram-negative organisms, including MDR strains of *P. aeruginosa* [4].



RX-P873

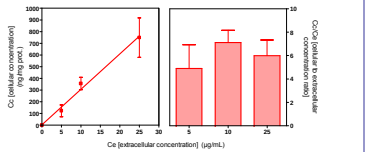
MW = 567.11
pKa_s = 8.9 – 9.6 – 10.5 – 12.1 (3+ at pH 7)
log P = 2.67
log $D_{pH\ 7}$ = -4.13
(calculated with Reaxys)

Aims of the study

- to determine the cellular accumulation of RX-P873 in THP-1 human monocytes
- to compare its extracellular and intracellular activity with that of ciprofloxacin and ceftazidime against susceptible and MDR *P. aeruginosa* using a pharmacodynamic model recently developed in our laboratory [1].

Results

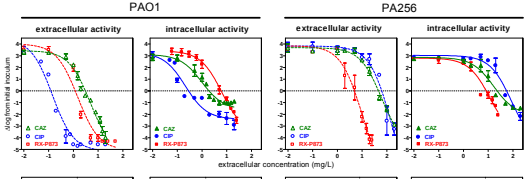
CELLULAR PHARMACOKINETICS: RX-P873 accumulates ~6-8 fold in THP-1 cells, with no sign of saturation for extracellular concentrations ranging from 5 to 25 mg/L.



Accumulation of RX-P873 in THP-1 cells after 2 h of incubation.
Left panel: apparent cellular concentration
Right panel: corresponding accumulation factors (cellular to extracellular concentration ratios)

SUSCEPTIBILITY TESTING: RX-P873 shows similar MIC against a fully susceptible and a MDR strain as well as against a series of SCVs.

Strains	Description	MIC (µg/mL)		
		CAZ	CIP	RX-P873
PAO1	Wild type	4	0.25	2
PA256	Clinical MDR	32	64	4
PA149	Clinical Small Colony variant	128	2	2
PA155	Clinical Small Colony variant	2	2	2
PA156	Clinical Small Colony variant	4	2	2
PA170	Clinical Small Colony variant	4	0.125	0.5
PA175	Clinical Small Colony variant	1	2	4
PA176	Clinical Small Colony variant	4	1	4
PA180	Clinical Small Colony variant	128	4	2
PA518	Clinical Small Colony variant	8	1	2



Extracellular (in broth) and intracellular (in THP-1 cells) activity of RX-P873 after 24 h of incubation, as compared to CAZ and CIP .
Extracellular and intracellular bacteria were exposed to increasing concentrations of antibiotics. We checked that RX-P873 did not cause marked cellular toxicity at the highest concentration tested (20 mg/L; LDH release ≤ 10 %). Data are expressed as changed (in log CFU) from the initial inoculum.
Upper panel: extracellular concentrations in mg/L
Lower panel: extracellular concentrations in multiples of the MIC.

PHARMACODYNAMICS:
- Extracellular and intracellular Cs are close to the MIC for all drugs (0.4 to 3-fold), making RX-P873 the most potent against the MDR strain.
- RX-P873 is the most effective against both extracellular and intracellular bacteria at 20 mg/L and was as or more effective than comparators at 10 x MIC.

Pharmacodynamic parameters calculated from Hill equations (R^2 > 0.90) fitted to data from Figure 2

Strains	Antibiotic[b]	Extracellular activity				Intracellular activity			
		C_s[a] (mg/L)[a]	C_s[a] (X MIC)	$E_{20mg/L}$[b]	E_{10XMIC}[c]	C_s[a] (mg/L)	C_s[a] (X MIC)	$E_{20mg/L}$[b]	E_{10XMIC}[c]
PAO1	CAZ	3.05	0.73	-3.70	-4.47	1.91	0.43	-1.38	-1.49
	CIP	0.15	0.49	< -4.50 [d]	< -4.50 [d]	0.29	1.14	-2.37	-1.98
	RX-P873	1.18	0.59	-4.43	-4.43	5.94	2.98	-1.97	-1.97
PA256	CAZ	45.85	1.43	1.56	< - 3.52 [e]	22.93	0.64	0.17	< -1.50 [e]
	CIP	62.25	0.97	2.29	< - 4.50 [e]	55.27	0.86	1.57	< -1.84 [e]
	RX-P873	4.91	1.22	-3.94	< -4.50 [e]	8.32	2.08	-1.48	-2.47

[a] extracellular conc. resulting in no apparent bacterial growth after 24 h of incubation; calculated from the Hill equation of the concentration-response curve (n= 3-6).
[b] change in CFU (in $\log_{10}$ units) at 24 h from the corresponding initial inoculum for an extracellular concentration of 20 mg/L (equal concentrations) ; calculated from the Hill equation of the concentration-response curve (n= 3-6).
[c] change in CFU (in $\log_{10}$ units) at 24 h from the corresponding initial inoculum for an extracellular concentration of 10xMIC (equipotent concentrations) ; calculated from the Hill equation of the concentration-response curve (n= 3-6).
[d] limit of detection (compared to the initial inoculum in broth [10^6 CFU/mL])
[e] the actual value could not be calculated because the maximal efficacy (BOTTOM plateau value of the Hill equation) was not reached at the highest concentration tested or because this concentration was higher than the maximal value tested.

Methods

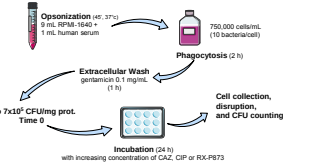
Bacterial strains : ATCC PAO1 (reference strain), PA256 (clinical MDR strain isolated from a patient suffering from hospitally-acquired pneumonia [5]); 8 small colony variants (SCV) strains isolated from cystic fibrosis.

Susceptibility testing : MICs determined by microdilution according to CLSI recommendations (CA-MHB; pH 7.4, 20 h)

Cellular accumulation : human THP-1 monocytes incubated during 2 h with the drug, pelleted by low speed centrifugation, washed in cold-PBS, pelleted again, collected in water, and dispersed by sonication. RX-P873 assayed by fluorimetry (λexc = 280 nm; λem = 450nm); cellular concentration calculated using as reference the sample protein content.

Concentration-kill curve studies in extracellular medium: 24 h incubation in 2 mL of CA-MHB using an initial inoculum of ~10^6 CFU/mL; plating and CFU counting after overnight incubation

Concentration-kill curve studies in intracellular medium: human THP-1 monocytes infected and treated as described in [1] and pictorially illustrated here below. Hill-Langmuir function (sigmoidal equation with Hill factor = 1) fitted to the data to calculate
- relative efficacy as $E_{20\ mg/L}$ or E_{10xMIC} (change of CFU for extracellular antibiotic concentration of 20 mg/L or 10x corresponding MIC)
- relative potency as C_s (concentration for which there is no apparent change in bacterial counts from the original inoculum).



Absence of cytotoxicity: assessed by measuring LDH release after 24 h of incubation with THP-1 cells.

Conclusions

- RX-P873 shows similar activity against normal and SCV phenotype of *P. aeruginosa* and is not affected by MDR phenotype.
- Despite its moderate accumulation in THP-1 cells, RX-P873 is potent and effective against extracellular and intracellular strains of *P. aeruginosa* whether fully susceptible to other antibiotics or harboring a MDR phenotype.
- Our data suggest a potential interest of RX-P873 for the treatment of resistant intracellular Gram-negative bacteria.